ISSN 0377–9335

ENTOMON

Vol. 24 December 1990 No. 4

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Kariavattom, Trivandrum, India 695581

ENTOMON

ENTOMON is a quarterly journal of the Association for Advancement of Entomology issued in March, June, September and December, devoted to publication of research work on various aspects of insects and other arthropods.

Address MS and all editorial correspondence to Managing Editor, ENTOMON, Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695581, India.

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ENTOMON 24(4): 297–306 (1999)
Article No. ent 4401

Isozyme Profiles in Relation to Ecological Status in Two Japanese Encephalitis Vectors, *Culex vishnui* and *Culex fuscocephala* (Diptera: Culicidae)

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ABSTRACT: Enzymes such as Esterase A and B (EST), alkaline phosphatase (APH), acid phosphatase (ACPH), malate dehydrogenase (MDH), aldehyde oxidase (ALDOX) and glucose-6-phosphate dehydrogenase (G-6-PD) were analysed in two populations of Japanese encephalitis (JE) vectors, *Cx. vishnui* and *Cx. fuscocephala*. Comparisons were made between population from an agricultural field at Mandya district and suburban areas of Mysore. Heterozygosity and allelic frequencies at different loci were calculated for each enzyme. The data has indicated significant variation between the two populations for EST alleles ($P < 0.01$). In mosquitoes, esterases are an important group of enzymes responsible for the development of insecticide resistance. As the JE vectors under study are subjected to an year round insecticidal selection pressure in Mandya district, the result can be correlated to the ecological status of the populations.

KEYWORDS: *Culex vishnui, Culex fuscocephala*, Japanese encephalitis, heterozygosity, polymorphism, resistance.

INTRODUCTION

Analysis of genetic variation by means of electophoretic techniques that came into large use since the late sixties, have been providing data of crucial importance. This approach has been proved particularly fruitful in the study of parasites and vectors (Bullini, 1985). Electrophoretic data allow evaluation of the genetic relationship existing between populations and taxa. Thus the gene enzyme analysis has been applied more and more to mosquitoes due to their medical importance and evolutionary interest (Cianchi *et al.*, 1985). It has been largely employed in the last twenty years in mosquitoes by many authors (Kitzmiller, 1976; Raymond *et al.*, 1987; Vaughan and Hemingway, 1995; Gopalan *et al.*, 1996, 1997; Revanna *et al.*, 1997).

Genetic variability studied in natural populations of mosquitoes showed that, about 40% of the loci are polymorphic. Estimations of the amount of variation in a population are measured by heterozygosity and polymorphism. Of late electrophoresis has been employed to monitor mosquito resistance to insecticides. The technique

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could be especially useful during epidemic outbreaks of arthropod borne diseases and mosquito control campaign. Moreover, electophoretic identification may prove useful in measuring the impact of pesticide applications on a particular species in different localities. Severini *et al.* (1993) have reported that non-specific esterase and acetyl cholin-esterase (AChE) are involved in OP resistance in *Cx. pipiens* from Italy. Further it was shown that the resistance to organophosphates in *Culex* mosquitoes is typically associated with increased activity of nonspecific esterases (Vaughan *et al.*, 1995). Thus many authors have reported that the esterases are responsible for the development of insecticide resistance in *Culex* and *Anopheles* mosquitoes (Callaghan *et al.*, 1991; Peiris and Hemingway, 1993; Failloux *et al.*, 1994; Whyard *et al.*, 1994; Vaughan and Hemingway, 1995; Qiao and Raymond, 1995; Wirth and Georghiou, 1996; Bourguet *et al.*, 1996; Guillemaud *et al.*, 1996; Gopalan *et al.*, 1996, 1997; Revanna *et al.*, 1997). In the light of such mounting information, the present investigation was undertaken on two populations of *Cx. vishnui* and *Cx. fuscocephala*, two JE vectors of local importance. These populations were screened from Mandya, the irrigated rice bowl of Karnataka state, and Mysore city outskirts. These species have registered differential insecticide susceptibility in our earlier studies employing WHO test kits (Vijayan *et al.*, 1993; Pushpalatha and Vijayan, 1994; Vijayan and Pushpalatha, 1997). In the light of the above knowledge the present investigation was undertaken in order to find out the genetic difference if any, between the two populations.

MATERIALS AND METHODS

Cx. vishnui and *Cx. fuscocephala* mosquitoes were collected from JE endemic villages of Mandya district and Mysore city areas. Both the species were reared in the laboratory. Either larvae or adults were used for different enzyme assays as per the reported activity of the enzyme.

Seven enzymes namely esterase A & B (EST-A,B), alkaline phosphatase (APH), acid phosphatase (ACPH), malate dehydrogenase (MDH), aldehyde oxidase (ALDOX) and glucose-6-phosphate dehydrogenase (G-6-PD) were analysed in the said populations. The experiments were conducted by using a vertical mini slab gel electrophoretic unit with gel length of 7 cm. The polyacrylamide gel electrophoresis was carried out following the techniques of Davis (1964) and Hegde and Krishnamurthy (1982).

One day old adults were used for detecting the enzyme profile of malate dehydrogenase and aldehyde oxidase as the activity of these enzymes would be high in adults. Fourth instar larvae were employed for analysing the esterase A and B, acid phosphatase, alkaline phosphatase and glucose-6-phosphate dehydrogenase. Specimens were homogenised with a solution of 0.1 ml of 40 per cent sucrose solution and a drop of 1.0 per cent bromophenol blue. The samples were centrifuged at 5,000 to 10,000 rpm for 3 to 5 minutes. 25 microlitre of the supernatant was kept above the large pore gel. Tray buffer was filled above the sample prior to the commencement of electrophoresis. The tray buffer was filled into the migration chambers and the electrodes were immersed. The electrophoresis was conducted at 40 °C using appropriate

electric current flow for 2 to 3 hours. At the end, the gel was removed and stained appropriately in order to expose the isozyme bands.

After the appearance of the bands, gels were fixed in 7.0 per cent acetic acid and later zymograms were taken to analyse the mobility of the bands. The commonest band appeared in both the populations of a species was designated as 1.00. Other bands with high mobility were designated with numbers in an increasing order (1.02, 1.04, 1.05, 1.06, etc.). Similarly the bands which had shown a low mobility were designated with numbers in a decreasing order from 1.00. The numbering has been done considering the distance moved by each band. Allelic frequency and heterogeneity at different loci were calculated following the methods of Sokal and Rohlf (1981) and Singh and Coulthart (1982).

RESULTS AND DISCUSSION

a. *Culex vishnui*

The electropherograms of the enzymes Esterase A & B, APH, ACPH, MDH, ALDOX and G-6-PD are provided in Fig. 1. Similarly allelic frequencies and G-values are given in Table 1. In the zymogram each band represents the product of one allele. The individuals with two or more than two bands were treated as heterozygotes. Different alleles of an enzyme are designated by using a number as super script for the abbreviated form of the enzyme in an ascending order. These numericals are provided in the order of decreasing mobility of each enzyme band. The electrophoretic profiles indicate that, Mysore and Mandya populations of the said species have 3 and 4 bands respectively for Esterase A. The Mysore population of the same species has two bands and the Mandya population possess three bands for Esterase-B. APH profile indicates two bands in both the populations. The ACPH enzyme has two alleles in Mysore and two in Mandya population. The profile of MDH enzyme revealed two alleles in both the populations. Isozyme pattern of ALDOX depicted two alleles for Mysore and three for Mandya population and for G-6-PD both the populations have two alleles. Details about the allelic frequencies in each population alongwith the heterozygosity per individual (Table 1) indicate significant variation between the two populations for Esterase A and B. This is important in view of the earlier findings where the Mandya populations have shown more tolerance to various insecticides (Pushpalatha and Vijayan, 1994; Vijayan and Pushpalatha, 1997; Pushpalatha and Vijayan, 1998).

b. *Culex fuscocephala*

Allelic frequencies and heterozygosity per individual for Esterase A & B, APH, ACPH, MDH, ALDOX, and G-6-PD enzymes in *Cx. fuscocephala* are furnished in the Table 2 and Fig. 2 depicts the zymogram pattern of these enzymes. Variants of esterase A shows 5 alleles in both the populations. For esterase B, three alleles were detected in Mysore and five alleles in Mandya population. APH locus of the said species has got two alleles in both the populations. Three bands were detected in Mysore as against four in Mandya population for ACPH enzyme. MDH locus of the

TABLE 1. Allelic frequencies of seven enzymes in *Cx. vishnui* populations

Locus	Electromorph	Mysore	Mandya	G-Value
Est. A	0.98		0.2931	
	1.00	0.5241	0.1667	16.571*
	1.02		0.178	
	1.04	0.054		
	1.07		0.362	
	1.23	0.422		
	H	0.546	0.723	
	N	83	87	
Est. B	0.96		0.500	
	1.00	0.545	0.188	13.421*
	1.03		0.313	
	1.23	0.455		
	H	0.496	0.617	
	N	66	80	
APH	0.97	0.500		
	1.00	0.500		
	1.10		0.467	
	1.14		0.533	
	H	0.500	0.498	
	N	60	60	
ACPH	0.74		0.211	
	0.77		0.106	
	0.96	0.558		
	1.00	0.441		
	1.01		0.141	
	1.03		0.542	
	H	0.494	0.630	
	N	68	71	
MDH	1.00	0.514	0.537	0.030
	1.03	0.486	0.463	
	H	0.499	0.497	
	N	70	54	
ALDOX	0.93	0.509		
	0.98		0.160	
	1.00	0.491	0.359	1.355
	1.02		0.481	
	H	0.499	0.614	
	N	56	78	
G-6-PD	0.98	0.500	0.500	
	1.00	0.500	0.500	
	H	0.500	0.500	
	N	69	72	

H - Heterozygosity per individual $P < 0.01$*
N - Number of individual sampled.

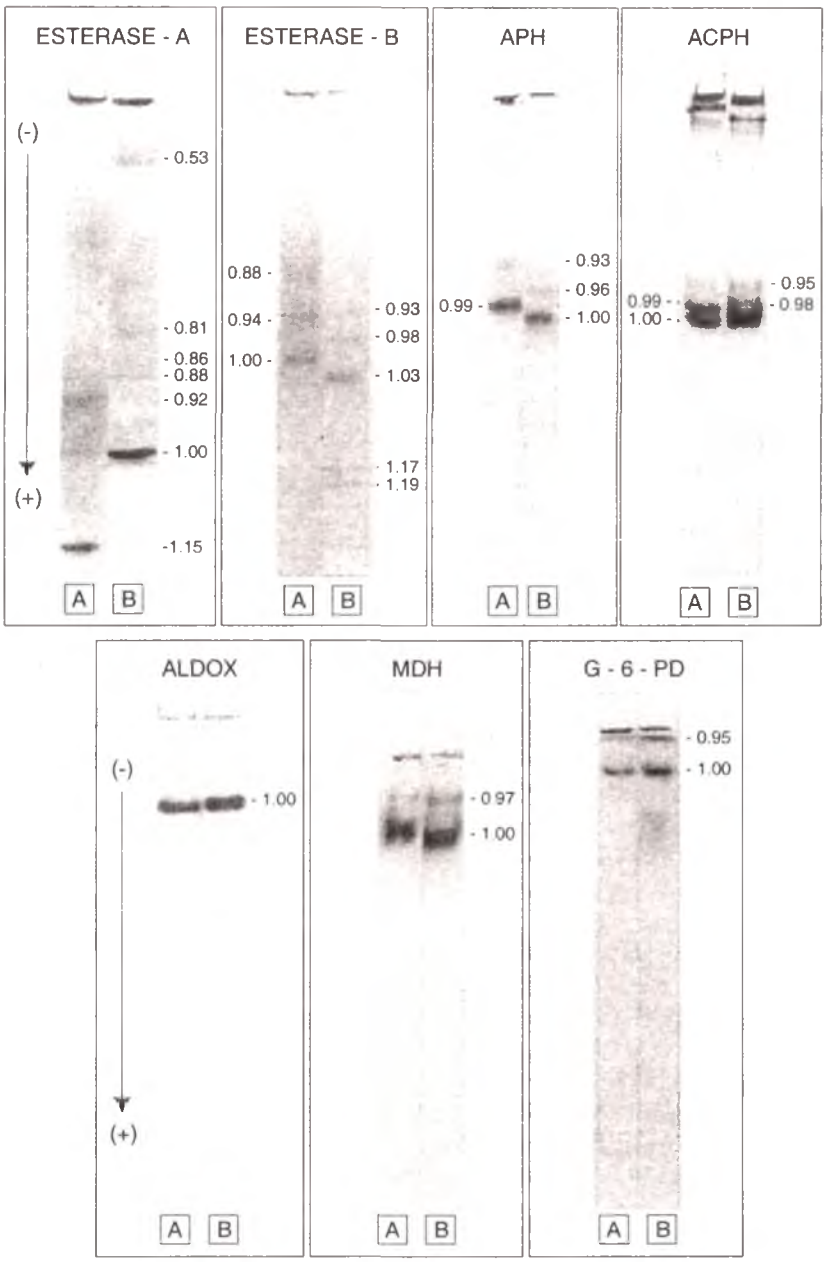

FIGURE 1. Isoenzyme profile of esterase A, B (EST-A,B), alkaline phosphatase (APH), acid phosphatase (ACPH), aldehyde oxidase (ALDOX), malate dehydrogenase (MDH) and glucose-6-phosphate dehydrogenase (G-6-PD) for Mysore (A) and Mandya (B) populations of *Cx. vishnui*.

said species has got two alleles in both the populations. Three bands were detected in Mysore as against four in Mandya population for ACPH enzyme. MDH locus of the said species has got two alleles in both the populations. The ALDOX enzyme profile indicates monomorphism in both the populations of the said species with single band. Two alleles were observed for G-6-PD enzyme in both the populations. In this species too significant variation between the two populations has been recorded for EST-A ($P < 0.01$).

Allozyme polymorphism as demonstrated through gel electrophoresis is a good biochemical parameter for evaluating the genetic differentiation existing within and between populations of a species or a complex or a group. The present analysis has been of immense value as a biochemical parameter to differentiate the two natural populations of two locally important JE vectors. It has been suggested that shifts in the alloenzyme content of a population could be identified for many environmental influences (Joslyn, 1984). The investigations by the authors revealed significant genetic differences between Mandya and Mysore populations of *Cx. vishnui* for esterase A & B, while in *Cx. fuscocephala* this difference was only for EST-A locus. The result is in line with the earlier findings of Revanna *et al.* (1997) on *Cx. tritaeniorhynchus* and *Cx. gelidus* from the paddy fields of Mandya and Mysore outskirts, where significant difference was seen for esterases in Mandya population. Further Ninge Gowda and Vijayan (1994) too found certain amount of variation between Mysore and Mandya populations of *Cx. quinquefasciatus* but not statistically significant. This may be because of the breeding habitats of this species being mainly urban drains. The present finding is important as Mandya is an endemic district for JE. Further this district, with vast stretch of rice field is irrigated having an year round insecticide pressure compared to Mysore city surroundings. Peiris and Hemingway (1990) too have revealed that, *Cx. quinquefasciatus* population of Srilanka had developed resistance against selected broad spectrum organophosphate insecticides due to selection pressure over many years. Organophosphorus and Carbamate insecticide pressure has created four types of resistance mechanism among *Culex* species. These are altered acetyl cholinesterase (AChE) which is insensitive to inhibition of the insecticides, oxidase and esterase based hydrolysis or sequestration and reduced penetration (Peiris and Hemingway, 1990). In many species strong resistance is due to two or more of these mechanisms (Villani and Hemingway, 1987). Chitra and Pillai (1985) have detected 15 to 16 esterase bands in *An. stephensi* from Delhi area where a malathion resistant strain showed 13 bands with 3 additional bands, 3a, 3b and 5a. However, a fenitrothion selected strain has shown only 10 bands. In a Saudi Arabian strain of *Cx. quinquefasciatus* resistance to temephos increased nine-fold during selection experiment (Amin and Peiris, 1990).

The population investigated presently are heterogeneous with regard to many alleles. In a population the heterogeneity may be with respect to different chromosome rearrangement or with regard to different alleles at a given locus. In mosquitoes, mainly esterases are responsible for the development of insecticide resistance. It has been reported that the increased esterase activity results in resistance in *Cx. pipiens* to

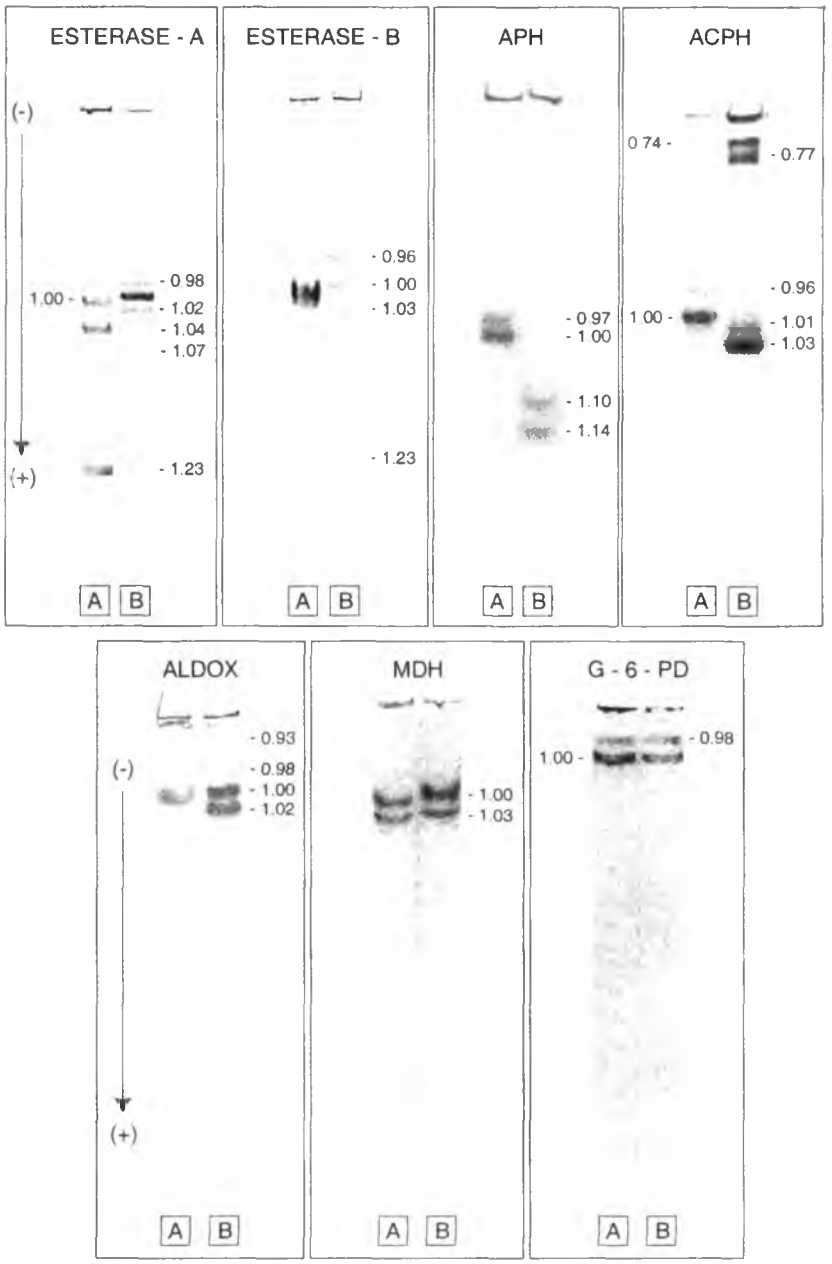

FIGURE 2. Isoenzyme profile of esterase A, B (EST-A,B), alkaline phosphatase (APH), acid phosphatase (ACPH), aldehyde oxidase (ALDOX), malate dehydrogense (MDH) and glucose-6-phosphate dehydrogenase (G-6-PD) for Mysore (A) and Mandya (B) populations of *Cx. fuscocephala*.

TABLE 2. Allelic frequencies of seven enzymes in *Cx. fuscocephala* populations

Locus	Electromorph	Mysore	Mandya	G-Value
Est. A	0.53		0.209	
	0.81		0.071	
	0.86	0.250	0.082	7.785*
	0.88	0.071	0.088	0.149
	0.92	0.173		
	1.00	0.143	0.549	22.083*
	1.15	0.363		
	H	0.750	0.635	
	N	84	91	
Est. B	0.88	0.403		
	0.93		0.484	
	0.94	0.113		
	0.98		0.016	
	1.00	0.484		
	1.03		0.139	
	1.17		0.082	
	1.19		0.279	
	H	0.591	0.662	
	N	62	61	
APH	0.93	0.456		
	0.96		0.413	
	0.99	0.544		
	1.00		0.587	
	H	0.496	0.485	
	N	68	63	
ACPH	0.95	0.304	0.278	0.006
	0.98		0.056	
	0.99	0.196	1.167	0.134
	1.00	0.500	0.500	0.000
	H	0.619	0.642	
	N	56	54	
MDH	0.97	0.485	0.471	0.014
	1.00	0.515	0.529	0.013
	H	0.499	0.498	
	N	65	68	
ALDOX	1.00	1.000	1.000	0.000
	H	0.000	0.000	
	N	68	67	
G-6-PD	0.95	0.492	0.500	
	1.00	0.508	0.500	0.004
	H	0.450	0.500	0.004
	N	61	65	

H - Heterozygosity per individual $P < 0.01$*
N - Number of individual sampled.

organophosphorus insecticides in many countries of Africa, Asia and North America. Thus measurement of allelic frequency in the field populations has become a clear method of choice for understanding the long and short term effects of insecticide use on population structure (Brogdon, 1989). That is, each population has got a genetic structure which make it susceptible or tolerant against various insecticides as the case may be. Both populations of the said species under study have shown polymorphism for all the enzymes tested except ALDOX of *Cx. fuscocephala*. This study therefore indicates the existence of genetic variability in natural population under different ecological situations.

ACKNOWLEDGEMENT

The authors wish to thank the Chairman, Department of Zoology, University of Mysore, for the facilities.

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(Received in March 1999; revised and accepted in November 1999)

ENTOMON 24(4): 307–313 (1999)
Article No. ent.24402

Effect of Precocene-II on the Nucleic Acid Contents in the Ovaries of *Chilo partellus, swinhoe*

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ABSTRACT: The nucleic acid contents in the ovaries of the normal larvae, pupae and the adults of *Chilo partellus* increased gradually, whereas in the Precocene II-treated larvae, the larval-pupal intermediates, precocious pupae and precocious adults, there was prominent decrease in DNA and RNA contents.

KEYWORDS: *Chilo partellus*, Precocene-II.

INTRODUCTION

Metamorphosis is characterized by the appearance of new structures capable of performing new physiological and biochemical functions. This requires formation of new tissues involving new proteins, DNA and RNA molecules. DNA and RNA are key biosynthetic pathways which operate actively in the larval stages and is thought to be an important preparatory mechanism for active metabolic functions to be carried out later by different organs during late larval development (Dean *et al.*, 1985). Endocrine signals appear as an important link between environment and various physiological and development events in insects (Denlinger, 1985; Steele, 1985; Steel and Davey, 1985; Truman, 1985). Juvenile hormone (JH) stimulates vitellogenin synthesis by the fat body (Koeppe and Offengand, 1976; Braun and Wyatt, 1992) and DNA synthesis by the ovary (Koeppe and Wellman, 1980). Decapitation arrests DNA synthesis and significantly retards protein synthesis in the ovary (John *et al.*, 1981; Garceq-MD *et al.*, 1989). In the light of the above observations an attempt has been made to elucidate the influence of Precocene-II on the RNA and DNA content in the ovaries during the development of *Chilo partellus*.

MATERIALS AND METHODS

Insect culture

The stem borer *Chilo partellus* is the most destructive pest of jowar, one of the main millet crops grown in arid zones. The above insect was reared on an artificial diet

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of sorghum leaf powder and chick pea flour at a temperature of 27 ± 1 °C and RH 65 ± 5%.

The insect passes through five larval instars and then pupates. Freshly moulted 2nd, 3rd and 4th instar larvae, freshly emerged pupae were treated topically on the abdominal region with 0.5–5 μg of Precocene-II dissolved in 1–2 μg of acetone with the help of Hamilton Microsyringe. More than 40 larvae and pupae were treated each time and the experiment was repeated with 3 batches of insects. Controls were treated with 1–2 μl of acetone. After the treatments, a suitable time gap of 10 min was given for the total absorption of Precocene-II and the larvae were transfered into the artifical diet.

Ovaries

The ovaries from late 4th & 5th instar larvae, pupae and female adults of control and the Precocene-II treated 2nd, 3rd, 4th, instar freshly emerged pupae, precocious adults emerged all were dissected under binocular microscope in freshly prepared Ringer's solution. Homogenate was prepared for the estimation of nucleic acids with 0.9% NaCl solution. The homogenate was centrifuged at 2500 rpm, and the supernatant was used for the estimation of RNA and the residue was used for the estimation of DNA.

RNA content of the ovaries was estimated by Orcinol reagent method, using synthetic RNA as the standard. DNA content of ovaries was estimated by the Dische's Diphenylamine reaction, using synthetic DNA as the standard.

RESULTS

RNA Estimation in ovaries of control insects

Larval stages

On the last day of the 4th instar (20 day old) larvae RNA content in the ovaries was 1.17 ± 0.019 mg/gm weight of the tissue. On the 2nd day of the 5th instar (22nd day) larvae the RNA content in the ovaries increased to 1.21 ± 0.019 mg/gm weight of the tissue and a further increase was observed on the 4th day of the 5th instar (24th day) of the larval period. The value was 1.27 ± 0.019 mg/gm weight of the tissue.

Pupal stage

RNA content in the ovaries of the freshly emerged pupa was 1.34 ± 0.026 mg/gm weight of the tissue. On the 2nd day of the pupal period, RNA content in the ovaries increased to 1.43 ± 0.019 mg/gm weight of the tissue. There was a further increase in the ovarian RNA content on the 4th day of the pupal period, 1.49 ± 0.019 mg/gm weight of the tissue. On the 6th day RNA content in the ovaries increased to 1.51 ± 0.06 mg/gm weight of the tissue.

Adults

RNA content in the freshly emerged female adult ovary was 1.55 ± 0.019 mg/gm weight of the tissue which increased to 1.60 ± 0.027 mg/gm weight of the tissue

TABLE 1. RNA content of the ovary of the *Chilo partellus* (expressed in mg/gm weight of the tissue) expressed as Mean, ±Standard Error

Age in days (8 days old treated)	Ovary RNA mg/gm wt of the tissue control	Age in days (8 days treated)	Ovary RNA mg/gm wt of the tissue Treated
4th Instar	$\overline{X} \pm$ S.E	4th Instar	$\overline{X} \pm$ S.E
20 days	1.17 ± 0.007	20 days	1.09 ± 0.005
5th instar		4th instar treated resultant 5th instar	
22 days	1.21 ± 0.008	22 days	1.07 ± 0.0057
24 days	1.27 ± 0.007	24 days	1.02 ± 0.009
pupae		pupae	
28 days	1.34 ± 0.010	25 days	0.97 ± 0.005
30 days	1.43 ± 0.007	27 days	0.92 ± 0.006
32 days	1.49 ± 0.008	29 days	0.85 ± 0.007
Adult		Adult	
35 days	1.55 ± 0.007	30 days	0.77 ± 0.010
37 days	1.60 ± 0.011	32 days	0.69 ± 0.010

Each value is the mean ± S.E of 6 individual observations, experimental values are statistically different from control with statistical significance at $P < 0.05$, *Not significant. $\overline{X}$-Mean of 6 values; S.E - Standard Error.

on the 2nd day. On the 4th day RNA content in the ovaries further increased to 1.64 ± 0.026 mg/gm weight of the tissue (Table 1).

Estimation in treated insects

There was a prominent decrease in the RNA content in the ovaries of the resultant, Precocene-II treated larvae, larval-pupal intermediates, precocious pupae, precocious adults of *Chilo partellus* when compared to that of the controls.

Larval stages

RNA content in the ovaries on the final day of the 4th instar (20 days old) larvae was 1.09 ± 0.013 mg/gm weight of the tissue. In Fourth instar Precocene- treated which emerged into 5th instar (22 days) RNA content in the ovaries decreased to 1.07 ± 0.014 mg/gm weight of the tissue. A further decrease in the RNA content was noticed in the ovaries on the 4th day of the 5th instar (24th day), which recorded 1.02 ± 0.023 mg/gm weight of the tissue.

Pupal stage

There was a slight decrease in the RNA level in the ovaries on the day of pupation. The value was 0.97 ± 0.013 mg/gm weight of the tissue. On the 2nd day of the pupal life RNA content in the ovaries decreased to 0.92 ± 0.015 mg/gm weight of the tissue. A further decrease in the RNA content in the ovaries, on the 4th day of the pupal period, where the recorded value was 0.85 ± 0.019 mg/gm weight of the tissue whereas in the control pupa the RNA content in the ovaries was 1.34 ± 0.026 mg/gm weight of the tissue.

Precocious adults

RNA level in the ovaries of freshly emerged precocious adult was 0.77 ± 0.026 mg/gm weight of the tissue. On the 2nd day of the adult life, RNA content in the ovaries decreased to 0.69 ± 0.026 mg/gm weight of the tissue whereas in the control adult the RNA content in the ovaries was 1.55 ± 0.019 mg/gm weight of the tissues. The precocious adult died on the 3rd day (Table 1).

Ovaries

DNA Estimation in ovaries of control insects

Larval stages

On the last day of the 4th instar (20th day) of the life cycle DNA content in the ovaries was 1.15 ± 0.019 mg/gm weight of the tissue. There was significant increases in the DNA content of the ovaries on the 2nd day of the 5th instar (22nd days old). The DNA content in the ovaries increased to 1.20 ± 0.013 mg/gm weight of the tissue.

Pupal stage

The DNA content in the ovaries of freshly emerged pupa was 1.35 ± 0.019 mg/gm weight of the tissue. On the 2nd day DNA content in the ovaries increased to 1.31 ± 0.019 mg/gm weight of the tissue. On the 6th day DNA content in the ovaries was 1.40 ± 0.08 mg/gm weight of the tissue.

Adults

The DNA content in the ovaries of freshly emerged female adult was 1.45 ± 0.019 mg/gm weight of the tissue which increased to 1.51 ± 0.019 mg/gm weight of the tissue on the 2nd day of the adult life. On the 4th day the DNA content in the ovaries further increased to 1.56 ± 0.06 mg/gm weight of the tissue (Table 2).

Estimation in treated insects

Larval stages

There was a steady decrease in the DNA content in the ovaries of the 2nd, 3rd, and 4th instar. Precocene-II treated resultant larval stages of *Chilo partellus* when compared to the controls.

TABLE 2. RNA content of the ovary of the *Chilo partellus* (expressed in mg/gm weight of the tissue) expressed as Mean, ±Standard Error

Age in days (8 days old treated)	Ovary RNA mg/gm wt of the tissue control	Age in days (8 days treated)	Ovary RNA mg/gm wt of the tissue Treated
4th Instar	$\overline{X} \pm$ S.E	4th Instar	$\overline{X} \pm$ S.E
20 days	1.17 ± 0.007	20 days	1.09 ± 0.005
5th instar		4th instar treated resultant 5th instar	
22 days	1.21 ± 0.008	22 days	1.07 ± 0.0057
24 days	1.27 ± 0.007	24 days	1.02 ± 0.009
pupae		pupae	
28 days	1.34 ± 0.010	25 days	0.97 ± 0.005
30 days	1.43 ± 0.007	27 days	0.92 ± 0.006
32 days	1.49 ± 0.008	29 days	0.85 ± 0.007
Adult		Adult	
35 days	1.55 ± 0.007	30 days	0.77 ± 0.010
37 days	1.60 ± 0.011	32 days	0.69 ± 0.010

Each value is the mean of ± S.E. of 6 individual observations, experimental values are statistically different from control with statistical significance at $P < 0.05$, *Not significant.

The DNA content in the ovaries on the last day of the 4th instar (20 day old) larvae was 1.04±0.013 mg/gm weight of the tissue. In Fourth instar Precocene- treated which emerged into 5^{th} instar (22nd day) of the life cycle the DNA content in the ovaries was 1.01 ± 0.019 mg/gm weight of the tissue. There was a decrease in the DNA content in the ovaries on the 4th day of the 5th instar (24 days old) larvae. The recorded value was 0.97 ± 0.013 mg/gm weight of the tissue.

Pupal stage

There was a decrease in the DNA level in the ovaries on the day of pupation. The recorded value was 0.92 ± 0.026 mg/gm weight of the tissue. On the 2nd day the DNA content in the ovaries further decreased to 0.87 ± 0.026 mg/gm weight of the tissue on the 4th day of the pupal life where as the DNA content in the ovaries of the control pupa was 1.25 ± 0.019 mg/gm weight of the tissue.

Precocious adults

DNA content in the ovaries of freshly emerged female precocious adults was 0.75 ± 02 mg/gm weight of the tissue. On the 2nd day of the adult life. DNA content in the ovaries decreased to 0.65 ± 0.026 mg/gm weight of the tissue whereas DNA content in the ovaries of the control adult was 1.45 ± 0.019 mg/gm weight of the tissue. The precocious adult died on the 3rd day (Table 2).

DISCUSSION

The results of this study demonstrate a gradual increase in the content of DNA and RNA of the ovaries coinciding with the increase in protein content during larval, pupal and adult development.

According to (Enesco and Leblond, 1962; Andres and Thummel, 1992) any increase in DNA content would reflect the growth. The amount of RNA determines the capacity of the cell to synthesize protein. The increase in the amount of RNA and DNA in the ovaries are probably associated with mitosis of ovarian tissues during maturation of the ovaries (Vandenberg, 1963; Telfer, 1965; Garceq-MD *et al.*, 1989). A decline in the RNA and DNA in the fat body was observed in the Precocene-II treated 2nd, 3rd and 4th instar resultant larvae, larval-pupal intermediates, precocious pupae and adults (Deena Vardhani, 1997).

Precocene-II has an apparently specific anti-allatotropic action (Nemec *et al.*, 1978; Pener *et al.*, 1978; Pedersen, 1979; Zhang *et al.*, 1993) similarly treatment of *Chilo partellus* larvae cause breakdown of the corpora allata, resulting in a precocious pupae and precocious adults, as is expected from an insect caused to be prematurely devoid of Juvenile hormone (Wyatt *et al.*, 1994). Both morphological observations and biochemical analysis of DNA, RNA and protein confirms the fact that Precocene-II deranges the development of *Chilo partellus* by blocking the synthesis and release of Juvenile hormone.

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(Received in April 1999; revised and accepted in July 1999)

ENTOMON 24(4): 315–322 (1999)
Article No. ent.24403

The Combined Efficacy of Dipel® and Malathion on the Development and Reproductive Potential of the Tropical Warehouse Moth, *Cadra cautella* (Walker) on Dried Mango

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ABSTRACT: Neonate larvae of the tropical warehouse moth, *Cadra cautella* (Walker) (Lepidoptera : Phycitidae) were subjected to low concentrations of Dipel® (commercial formulation of *Bacillus thuringiensis* var. *kurstaki*), the organophosphate, malathion (Malaton®) and Dipel® plus malathion. It was observed that the agents significantly reduced the pupal and adult recovery, developmental periods and reproductive potential of the insect.

KEYWORDS: Dipel®, Malathion, *Cadra cautella*.

INTRODUCTION

Bacillus thuringiensis Berliner (*B. t.*) is a Gram-positive, aerobic bacterium widely distributed in soil and insect-rich environments (Bart and Marnix, 1992). It is known to produce insecticidal crystal proteins during sporulation which manifest their action when ingested by lepidopteran, dipteran and coleopteran larvae depending upon the strain (Fast, 1981; Luthy *et al.*, 1982; Aronson *et al.*, 1986; Whiteley and Schnepf, 1986; Knowles and Ellar, 1987; Hofte and Whiteley, 1989; Carroll, 1990; Gill *et al.*, 1992; Koni and Ellar, 1994). *B. thuringiensis* is a very safe and the most widely used of the biopesticides (Wilcox, 1987).

The indiscriminate use of chemical pesticides and high energy input agriculture bring them severe problems, viz. pest resistance; hazards to man, domestic animals and beneficial arthropods; biomagnification, etc., which have led to the search for alternative pest management strategies. One component is the harnessing of pathogenic microorganisms with sublethal concentrations of chemicals encompassing the dual benefits of getting the desired degree of plant protection yet rendering the environment clean. The effects of *B. thuringiensis* with organophosphorous and several other insecticides for suppressing insect pest populations have been determined

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(Chen *et al.*, 1974; Creighton and McFadden, 1974; Dabi *et al.*, 1978, 1988; Habib and Garcia, 1981; Richter and Fuxa, 1984; Kramer *et al.*, 1985).

Mango (*Mangifera indica* L.) is a nutritious and delicious fruit grown in many tropical countries including Bangladesh, and a substantial quantity of mango is stored in a dried condition for future use. The tropical warehouse moth, *Cadra cautella* (Walker) is a serious pest of several stored commodities like cereals, nuts, dried fruits, etc.

Pramanik *et al.* (1994) recently observed that *B. t.* var. *kurstaki*-Malaton® concentrations produced mostly synergistic and sometimes additive effects on larval mortality in *C. cautella*. The present investigation reports the effects of Dipel® (*Btk*) and Malathion (Malaton®) combinations, both at sublethal concentrations, on the development and reproductive potential of *C. cautella* on dried mango.

MATERIALS AND METHODS

The test insects (*C. cautella*) were collected as larvae from a Government Warehouse in Rajshahi and were reared on groundnuts (*Arachis hypogaea* L.) to get adults. Moths of the opposite sexes were paired and mated females were utilized for oviposition.

The experimental sample Dipel®, a commercial formulation of *B. t.* var. *kurstaki* (16,000 International Units of Potency/mg., 3.2% a.i.), was supplied by the Abbott Laboratories, USA, in the form of wettable powder. Malaton® (Malathion) is an organophosphate manufactured by the ICD group, USA and supplied by the Chemsfil (Bangladesh) Ltd. It is a colourless water soluble liquid with a low mammalian toxicity and is a contact-stomach poison. Green mangoes were collected from the local market, cut into thin slices, dried in the sun and were preserved in air-right plastic containers for future use.

The concentrations, viz. Dipel® 10, Malaton® 2, Dipel® 10 + Malaton® 2, Dipel® 20, Dipel® 20 + Malaton® 2, Dipel® 30, Dipel® 30 + Malaton® 2, Dipel® 40, and Dipel® 40 + Malaton® 2 ppm; and Malaton® 2, Dipel® 10, Malaton® 2 + Dipel® 10, Malaton® 4, Malaton® 4 + Dipel® 10, Malaton® 8, Malaton® 8 + Dipel® 10, Malaton® 16, and Malaton® 16 + Dipel® 10 ppm were prepared by mixing the required amounts of the biopesticide and the insecticide in distilled water. Mango slices were treated by spraying with an hand automizer. Neonate *C. cautella* larvae were transferred to food treated by spraying with an hand automizer. Neonate *C. cautella* larvae were transferred to food treated with *Btk*, Malaton® and *Btk*-Malaton® concentrations. Untreated checks were raised on dried mango sprayed with distilled water only. Two concentration schedules were employed : a constant insecticide concentration with variable pathogen concentrations, and a constant pathogen concentration with variable insecticide concentrations. Fifty larvae were used in each treatment and the experiments were replicated thrice in petridishes (9.5 cm diam.).

TABLE 1. Effect of Dipel®–Malaton® concentrations on the pupal and adult recoveries in *C. cautella* (N = 150)

	Dipel® Conc. (ppm)	Malaton® Conc. (ppm)	Pupal Recovery (%)				Adult Recovery (%)			
			Dipel®	Malaton®	Dipel®+ Malaton®	*d-value	Dipel®	Malaton®	Dipel®+ Malaton®	*d-value
	10	2	56.00	66.00	29.33	11.37	52.66	62.00	26.00	10.99
A	20	2	48.00	66.00	20.66	14.25	44.66	62.00	17.33	15.40
	30	2	42.00	66.00	12.66	17.50	38.00	62.00	9.33	19.60
	40	2	34.00	66.00	1.33	25.90	30.00	62.00	00.00	27.55
	10	2	54.66	65.33	30.66	10.85	52.00	62.66	27.33	10.36
B	10	4	54.66	50.66	18.66	14.71	52.00	47.33	16.66	13.87
	10	8	54.66	43.33	12.00	17.66	52.00	40.00	10.00	16.66
	10	16	54.66	32.00	00.00	27.55	52.00	28.00	00.00	23.76

Control pupal and adult recoveries were 82.66 and 78.66% respectively, A = Malaton® concentration constant, B = Dipel® concentration constant, *d = Standardized normal deviate value.

TABLE 2. Effect of Dipel®-Malaton® concentrations on the developmental periods of *C. cautella*

Concentration schedule	Concentrations (ppm)	Larval period (days) Mean ± SD	Pupal period (days) Mean ± SD
	O(Control)	26.83 ± 4.20^{hi}	7.85 ± 2.63^{e}
	Dipel®, 10	28.21 ± 4.66^{efgh}	7.99 ± 2.87^{e}
	Malaton®, 2	26.92 ± 4.06^{hi}	7.94 ± 2.49^{e}
	Dipel® + Malaton®, 10 + 2	29.14 ± 4.94^{cdef}	8.53 ± 2.01^{d}
A	Dipel®, 20	28.86 ± 4.61^{defg}	8.39 ± 2.43^{d}
	Dipel® + Malaton®, 20 + 2	29.93 ± 4.63^{ab}	8.86 ± 2.74^{b}
	Dipel®, 30	30.21 ± 4.56^{bcd}	8.72 ± 2.67^{c}
	Dipel® + Malaton®, 30 + 2	31.63 ± 5.60^{ab}	9.10 ± 2.83^{b}
	Dipel®, 40	31.00 ± 4.64^{abc}	9.01 ± 3.22^{a}
	Dipel® + Malaton®, 40 + 2	32.38 ± 5.94^{a}	—
	O(Control)	26.87 ± 4.12^{s}	7.85 ± 2.63^{r}
	Malaton®, 2	27.03 ± 5.22^{rs}	7.92 ± 2.55^{qr}
	Dipel®, 10	28.17 ± 4.63^{pq}	7.96 ± 2.48^{pq}
	Malaton® + Dipel®, 2 + 10	29.21 ± 4.38^{lmnp}	8.45 ± 2.62^{n}
B	Malaton®, 4	27.88 ± 4.41^{qr}	8.05 ± 2.83^{p}
	Malaton® + Dipel®, 4 + 10	29.83 ± 5.04^{klm}	8.73 ± 2.67^{m}
	Malaton®, 8	29.42 ± 4.65^{lmn}	8.66 ± 2.85^{m}
	Malaton® + Dipel®, 8 + 10	30.66 ± 4.39^{k}	9.02 ± 2.79^{k}
	Malaton®, 16	30.17 ± 3.89^{kl}	8.89 ± 2.38^{l}
	Malaton® + Dipel®, 16 + 10	—	—

A = Malaton® concentration constant, B = Dipel® concentration constant. Means followed by the same letter are not significantly different at 1% level (DMRT)

TABLE 3. Effect of Dipel®-Malaton® concentrations on the fecundity and egg-viability of *C. cautella*

Concentration Schedule	Concentration (ppm)	Fecundity Mean ± SD (No.)	Egg-viability (%) Mean ± SD	Variance Ratio, F
	O(Control)	266.14 ± 18.85(15)	96.28 ± 3.14	
	Dipel®, 10	254.00 ± 9.61(15)	92.14 ± 3.56	
	Malaton®, 2	253.47 ± 12.13(15)	93.68 ± 3.84	
	Dipel® + Malaton®, 10 + 2	217.54 ± 6.79(12)	90.56 ± 4.12	
A	Dipel®, 20	241.14 ± 8.81(15)	89.77 ± 4.45	[a]46.29***
	Dipel® + Malaton®, 20 + 2	194.91 ± 10.56(8)	85.63 ± 4.38	[b]28.66***
	Dipel®, 30	234.43 ± 7.37(15)	84.72 ± 4.27	
	Dipel® + Malaton®, 30 + 2	178.56 ± 6.73(5)	79.03 ± 4.86	
	Dipel®, 40	225.67 ± 7.46(14)	78.87 ± 5.13	
	Dipel® + Malaton®, 40 + 2	—	—	
	O(Control)	266.14 ± 18.85(15)	96.28 ± 3.14	
	Malaton®, 2	251.87 ± 10.14(15)	93.46 ± 4.10	
	Dipel®, 10	253.27 ± 8.57(15)	92.38 ± 4.72	
B	Malaton® + Dipel®, 2 + 10	218.40 ± 6.10(13)	90.88 ± 3.98	
	Malaton®, 4	238.73 ± 7.47(15)	90.16 ± 5.03	[a]29.34***
	Malaton® + Dipel®, 4 + 10	197.92 ± 7.20(8)	86.77 ± 4.69	[b]12.53***
	Malaton®, 8	224.69 ± 5.89(15)	87.09 ± 4.43	
	Malaton® + Dipel®, 8 + 10	181.67 ± 11.40(5)	80.83 ± 4.07	
	Malaton®, 16	207.64 ± 9.75(14)	79.03 ± 5.12	
	Malaton® + Dipel®, 16 + 10	—	—	

A = Malaton® concentration constant, B = Dipel® concentration constant. [a]For fecundity, [b]For egg-viability, ***$P < 0.00$

Larvae were regularly checked for pupation and the larval period was noted. Pupae were sexed by the microscopic observation of the genital aperture lying on the midpostero-ventral line. They were kept in separate beakers for adult eclosion and the pupal period was recorded. The pupal and adult recoveries (%) were also recorded. Freshly emerged moths of the opposite sexes were allowed to mate and the mated females were retained in 500 ml beakers for egglaying. The oviposition by the moths was recorded and the eggs were observed for their viability.

The whole experiments were conducted at a mean room temperature of 29 ± 1°C.

RESULTS AND DISCUSSION

The effects of Dipel® - Malaton® concentrations on pupation and adult emergence in *C. cautella* are shown in Table 1. The results reveal that all the treatments significantly reduced the production of progeny in comparison to untreated controls, and their effect was more pronounced when higher concentrations were used. Faruki (1993) also found a significant reduction in pupation and adult emergence in *C. cautella* followed by treatments with *Btk*, Fenom® (a pyrethroid) and *Btk*-Fenom® concentrations.

In the present study the larval period in pathogen-insecticide treated insects were lower than those in the individual treatments of pathogen and insecticide concentrations (Table 2). These were also found to be true for the pupal period. El-Sebae *et al.* (1990) observed a retarded development in the Mediterranean fruitfly, *Ceratitis capitata* due to joint action of *B. thuringiensis* and conventional insecticides. Faruki (1993) also recorded significant reductions in developmental periods in *C. cautella* due to *Btk*, Fenom® and *Btk*-Fenom® treatments.

Conney *et al.* (1966) reported that organophosphate insecticides inhibit the hydroxylation process of some steroids. Ecdysone is a steroid which plays an important role in the moulting process in insects. If the insecticides in question block the hydroxylation process, there may not be enough of this hormone necessary for moulting and this may cause prolongation of the larval period.

The fecundity and egg-viability of the *C. cautella* females resulting from various treatments were significantly reduced in comparison to the control insects and in this regard, combined concentrations produced synergistic effects on these parameters (Table 3). Faruki (1993) working with *C. cautella* observed significant reductions in the fecundity and egg-viability of the insects when treated with the synthetic pyrethroid, Fenom® and *B. t.* var. *kurstaki*, applied singly and in combination.

Results showed that the effect of treatments was greater when the pathogen concentrations were variable and the insecticide concentration was kept constant.

It has been observed that the synergism may be due to physiological stress, reduction of phagocytic haemocytes caused by the chemical insecticides, or inhibition of the insect's detoxification mechanisms by the microorganisms (Telenga, 1957, 1958; Benz, 1971; Vail *et al.*, 1972; Boman, 1981).

The use of low concentrations of insecticides in combination with insect pathogens is a promising approach to the control of insect pests where there is synergism.

The present study shows that the combined application of insecticide and pathogen is a feasible method to substantially reduce the quantity of chemicals used in pest management programmes and their subsequent adverse impact on the ecosystem and natural activity. Dried mangoes, as human foods, need utmost protection against contamination with harmful agents like chemical pesticides. Thus the present study shows that *C. cautella* can be effectively controlled with very low concentrations of the organophosphate when used in combination with the bacterium. This offers a bright prospect of further research.

ACKNOWLEDGEMENTS

The authors are most grateful to the Chairman, Department of Zoology, Rajshahi University, for giving the necessary laboratory facilities, and to the Abbott Laboratories, USA and the ICD group, USA, for kindly supplying Dipel® and Malathion® respectively.

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(Received in January 1999; revised and accepted in July 1999)

ENTOMON 24(4): 323–330 (1999)
Article No. ent.24404

Histomorphological Studies on the Midgut of *Iphita limbata* Stal (Heteroptera: Pyrrhocoridae)

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ABSTRACT: The midgut which is the longest part of the alimentary canal of *Iphita limbata* is differentiated into five morphologically and histologically distinct regions—the first, second, third, fourth and fifth ventriculi. The midgut wall is formed of an outer longitudinal and inner circular muscle layers, basement membrane and epithelium. In the first ventriculus, the anterior and posterior regions are histologically different. In the middle and posterior regions, ventricular wall is formed of broader and narrower epithelial regions. Epithelium consists of mainly columnar cells, few cuboidal cells and regenerative cells. The histological features indicate that the first ventriculus and the anterior region of the second ventriculus are mainly secretory in function. The third ventriculus is both secretory and absorptive in function. The fourth ventriculus is mainly absorptive in function. Fifth ventriculus is a short bulbous portion and has no role in digestion and absorption.

KEYWORDS: *Iphita limbata*, midgut cells.

INTRODUCTION

The midgut of insects comprises the longest and functionally most important part of the digestive tract, dealing primarily with the digestion of food stuffs and the absorption of nutrients. Marks (1959) gives a comparative account of midgut epithelium of five water bugs. Parsons (1959) gives an account of midgut of the aquatic Heteroptera with emphasis on histological aspects, which is of immense value in understanding the histology and secretion of midgut epithelium in Heteroptera. But the studies on the members of Phyrrhocordiae is limited to a few species (Khanna, 1964; Kurup, 1964; Muraleedharan, 1983; Vijayakumar and Mohamed, 1991). Considering the lack of sufficient information on the alimentary canal of species belonging to the family Pyrrhocordiae, the present study has been carried out on the midgut of *Iphita limbata*.

MATERIALS AND METHODS

The experimental insects were collected from the field. In the laboratory they were kept in cages and were fed with banana. Adults were removed from the stock

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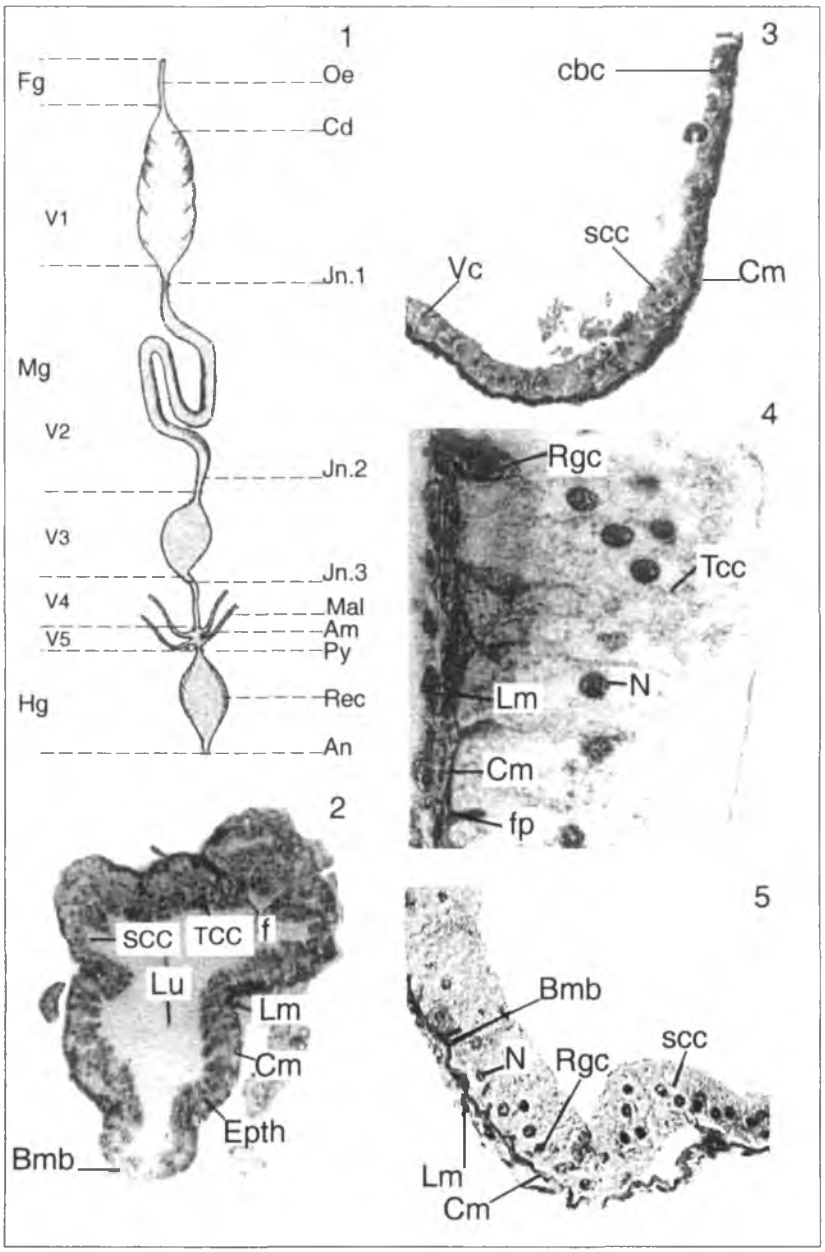
culture, kept in separate containers and were fed with banana for 20–30 minutes when they attained well-fed condition. These well-fed insects were starved for 3 days. These insects which were considered as normal were used for the histomorphological studies. They were anaesthetised and dissected to recover the alimentary canal. For histological studies, paraffin blocks of the alimentary canal was cut into 5–7 μm thick serial sections by a rotary microtome and sections were stained with Delafield's haematoxylin and counter stained with eosin.

RESULTS AND DISCUSSION

Morphology

The midgut of *I. limbata* forms the longest part of the alimentary canal and it measures slightly longer in females than in males as reported by Khanna (1964) in *Dysdercus koenigii*. It measures about 4.5–5 cm and about 5–6 cm in length in male and female respectively. Anteriorly the oesophageal valve demarcates it from the foregut and at the posterior end the pylorus separates it from th hindgut. The midgut or ventriculus of almost all the heteropteran insects is differentiated into three or four distinct regions (Goodchild, 1963; Srivastava and Singh, 1966; Mall, 1979; Muraleedharan, 1983; Pakrutty and Mohamed, 1989). The midgut of *I. limbata* exhibits five distinct regions (Fig. 1), the first (V_1), second (V_2), third (V_3), fourth (V_4) and fifth (V_5) ventriculi. The junction between the adjacent ventriculi is marked by constriction except between the fourth and fifth ventriculi.

The first ventriculus forms the anterior one third portion of the midgut. It is an elongated wide sac like region. Its anterior part forms a bulbous structure called cardia in which hangs oesophageal valve. The wall of first ventriculus is thin and transparent and the anterior region is convoluted due to many transverse folds. Air bubbles are invariably present in the lumen of this region. The second ventriculus is the longest tubular part of the midgut. After its origin it runs forward along the left ventrolateral margin of the first ventriculus till it reaches the second abdominal segment, crosses to the right, passes beneath the first ventriculus and runs backward reaching upto fifth abdominal segment. In females the second ventriculus is longer than that of males. The third ventriculus is a short dilated sac, located between the fifth and sixth abdominal segment. It is a thin-walled retention chamber lying ventral to the first ventriculus and encircled by the coiled second ventriculus. Its lumen is constantly filled with a reddish brown or black viscuous substance irrespective of whether the insect is well-fed or starved. The fourth ventriculus is a narrow and short tubular region and lies in the sixth abdominal segment. It joins the fifth ventriculus without any constriction. The fifth ventriculus is a small part of the midgut in between the fourth ventriculus and hindgut. it forms a bulbous portion where there is a pair of slightly swollen dorso-lateral outgrowths, the ampullae, each one of which receives a pair of Malpighian tubules. Each pair empties into ampullae independently. The morphological features of *I. limbata* are in accordance with the findings of Khanna (1964) in *D. koenigii* and Muraleedharan (1983) in *D. cingulatus* except that the midgut of *I. limbata* shows



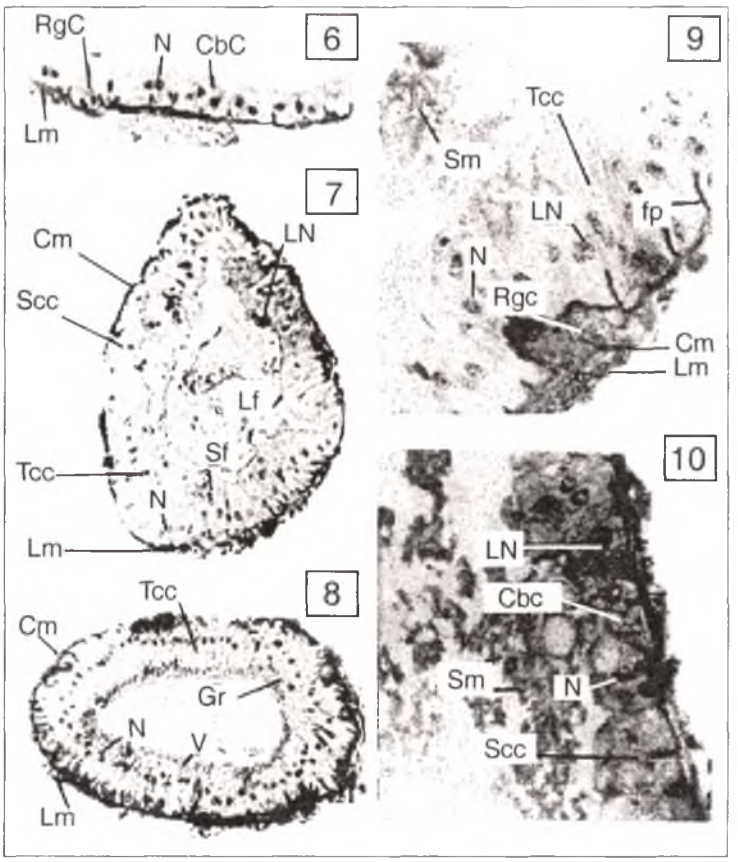
FIGURES 1–6. 1: The diagrammatic representation of the external anatomy of the alimentary canal of *Iphita limbata* 2: T.S. of the anterior region of the first ventriculus (100 χ) 3: A portion of the middle region of the first ventriculus showing large nucleus (100 χ) 4: An enlarged portion of the middle region of the first ventriculus showing tall columnar cells (750 χ) 5: A portion of T.S. of the middle region of the first ventriculus showing short columnar cells (300 χ) 6: A portion of T.S. of the middle region of the first ventriculus showing cuboidal cells (300 χ).

five divisions. There is no external demarcation between the fifth ventriculus and the hindgut.

Histology

The midgut epithelium of *I. limbata* is typically gymnocerate type (Goodchild, 1952; Khanna, 1964; Kurup, 1964; Mall, 1979; Muraleedharan, 1983; Singh and Sharma, 1987). Histologically the midgut wall is composed of a single layered epithelium (Fig. 2, Epth) basement membrane (Bmb) surrounded by an inner layer of circular (Cm) and an outer layer of longitudinal muscle (Lm) fibres. Peritrophic membrane is not traceable on the inner surface of the epithelium. Differences in the histological structure are observed at various regions of the midgut. The muscle layers are well developed in different ventriculi of *I. limbata* as observed in *D. koenigii* (Khanna, 1964). The epithelium is arranged on a thick basement membrane which exhibits short finger shaped projections into the cell layer (Figs 4, 6 and 9) except in fifth ventriculus. The number of projections formed by the basement membrane is fewer in fourth ventriculus when compared with other ventriculi. No information is found available on this structure in the previous studies on species belonging to Pyrrhocoridae. Three regions are recognized on the basis of differences observed in the epithelium of the first ventriculus of *I. limbata*. Two regions have been reported in *Cimex* (Cragg, 1914), *Chrysocoris stollii* (Srivastava and Singh, 1966) and in *B. cruciferarum* (Mall, 1979). The epithelium in the anterior region of the first ventriculus (Fig. 2) differs from the posterior region (Fig. 3). In the anterior region the folds formed by the epithelium are large and project into the lumen. It consists of tall columnar (TCC) and short columnar cells (SCC) whereas in the posterior region no folds are formed by the epithelium which is formed of short columnar and cuboidal cells (CbC). In the middle region of the first ventriculus the cells of one side is broader with tall columnar cells (Fig. 4) and of the other side is narrower with short columnar (Fig. 5) and cuboidal cells (Fig. 6). Towards the posterior region the epithelium is formed of shorter cells consisting of short columnar cells in the broad region and cuboidal cells in the narrow region. In the anterior part of the second ventriculus (Fig. 7) the epithelium formed of columnar cells exhibits a few infolding while in the posterior part (Fig. 8) infoldings are not observed. The epithelium of the third ventriculus (Figs 9 and 10) is smooth except for a few shorter folds formed all along its length. In addition to tall and short columnar cells, a few cuboidal cells are tall columnar in the fourth ventriculus (Fig. 11) and distinct fold formation is not seen. They are club shaped with long lobe like tips projecting into the lumen. In the fifth ventriculus (Fig. 12) the epithelium is folded and consists of tall columnar cells. It has been reported in *S. macilentus* (Singh and Sharma, 1987) in the anterior part of the first midgut that the epithelium is produced into many large folds whereas in the second and third midguts a few occasional low folds are present all along its length. In the case of *Chrysocoris purpureus* (Bhaskaran *et al.*, 1969) the epithelium of the first and third midguts are provided with epithelial folds.

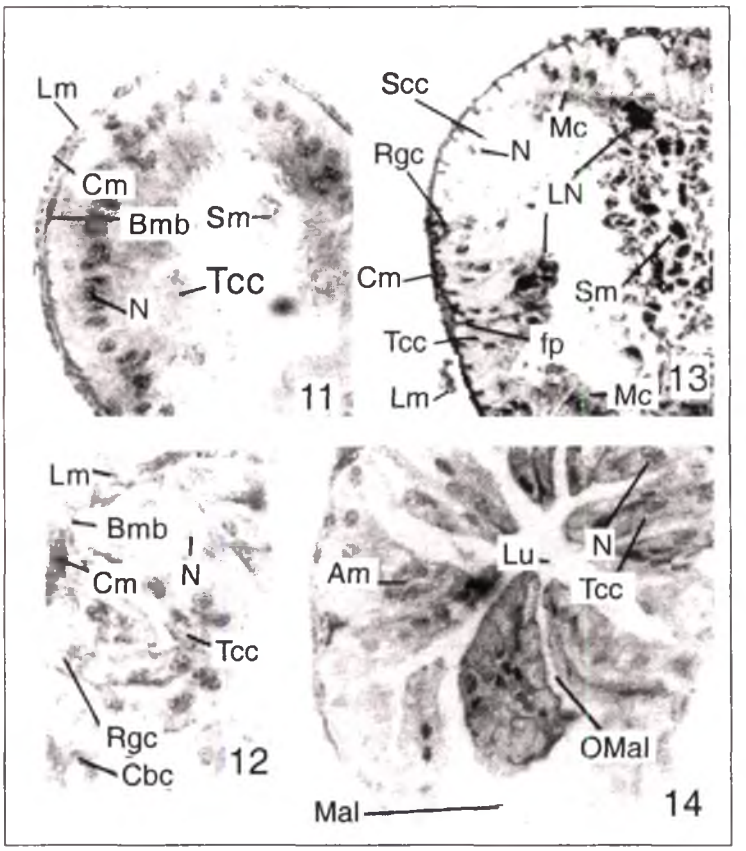
The cells in the anterior and middle regions of the first ventriculus are with uniformly distributed granular cytoplasm whereas in the posterior region some cells



FIGURES 7– 10. 7: T.S. of the anterior region of the second ventriculus (200 χ) 8: T.S. of the posterior region of the second ventriculus (200 χ) 9: A portion of T.S. of the third ventriculus showing tall columnar cells (250 χ) 10: A portion of T.S. of the third ventriculus showing short columnar and cuboidal cells (250 χ).

are vacuolated and others are with granular cytoplasm. The cytoplasm of the tall columnar cells of the second ventriculus is richly granular and vacuolated. Some cells of the third ventriculus are vacuolated while others contain uniformly distributed cytoplasm. In the case of fourth ventriculus, the cytoplasm of the cell is uniformly distributed without granules except in a few cells where vacuoles are seen at the tip. The cells in the fifth ventriculus have uniform cytoplasm.

The cells in the anterior and middle regions of first ventriculus are uni, bi or multinucleated, but in the posterior region, some cells are provided with large nucleus (LN). In the second ventriculus, both uni and binucleated cells occur. At certain cells the nuclei fuse to form large nucleus which is located at the cell tip and similar stained structures are also seen in the lumen (Fig. 13). The cells in the



FIGURES 11– 13. 11: A portion of T.S. of the fourth ventriculus (300 χ) 12: A portion of T.S. of the fifth ventriculus (400 χ) 13: An enlarged portion of T.S. of the middle region of the second ventriculus showing secretory activity (400 χ) 14: A portion of T.S. of the ampulla showing the opening of the Malpighian tubules (400 χ)

Am – Ampulla of the Malpighian tubules, An – Anus, Bmb – Basement membrane, Cd – Cardia, Cm – circular muscle, Cbc – Cuboidal cell, Epth – Epithelium, Fg – Foregut, Fp – Finger-like processes of the basement membrane, Gr – Granules, Hg – Hindgut, Jn.1 – Junction 1 between first and second ventriculi, Jn.2 – Junction 2 between second and third ventriculi, Jn.3 – Junction 3 between third and fourth ventriculi, Lf - Long folds, Lm – Longitudinal muscle, LN – Large nucleus, Lu – Lumen, Mal – Malpighian tubule, Mc – Merocrine cell, Mg – Midgut, N – Nucleus, Oe – Oesophagus, OMal – Opening of Malpighian tubule, Py – Pylorus, Rec – Rectum, RgC Regenerative cell, Sm – Secretory material, SCC – Short columnar cell, Sf – Short folds, TCC 0 Tall columnar cell, V – Vacuole, V_1 – First ventriculus, V_2 – Second ventriculus, V_3 – Third ventriculus, V_4 – Fourth ventriculus, V_5 – Fifth ventriculus, Vc – Vacuolated cell.

third ventriculus are uni or bi nucleated and the nuclei take very little stain. It is suggested that it may be due to degeneration. In the fourth ventriculus the nuclei of the cells are large, round and are seen at the centre of the cell. The cells in the fifth ventriculus is uni or binucleated but the nucleus is located towards the tip of the folds. Yanai and Iga (1956) found that the presence of binucleate cells in the

midgut epithelium is characteristic of gymnocerate whereas cryptocerate midgut cells are always uninucleate. Goodchild (1966) opined that the uninucleate midgut cells are abundant in highly carnivorous Cryptocerata and binucleate midgut cells are usually found in phytophagous Hemiptera. Singh and Sharma (1987) pointed out that the holocrine cells in the midgut are multinuclear while merocrine cells are uninuclear.

Regenerative cells are present in the first ventriculus mostly as single cell but rarely two cells are present at a locus whereas in the second and third ventriculi, the presence of single regenerative cells is a rare occurrence, but they are usually present in a group of three or four cells. There is a great reduction in the number of regenerative cells in the fourth and fifth ventriculi. In *B. cruciferarum* (Mall, 1979) regenerative cells are present singly or in a group of 2–3 cells. It has been reported that in phytophagous Pentatomidae there are a few regenerative cells while in the highly carnivorous cryptocerate nidi are abundant (Goodchild, 1966).

The presence of vacuolated cells with contents releasing into the lumen indicates secretion of material. The presence of such cells in the first, second and third ventriculi of midgut of *I. limbata* suggests that these regions are involved in intense secretory activity. The occurrence of regenerative cells as single or in groups also supports this view. The distinct histological features seen in the epithelial cells of the posterior region of the second ventriculus and fourth ventriculus and the reduction in the regenerative cells in these regions indicate that they are scarcely secretory and mainly absorptive. Intense secretory activity has been reported in the second region of the midgut of *C. purpureus* (Bhaskaran *et al.*, 1969).

The fifth ventriculus of *I. limbata* is very short swollen or globular region in between the fourth ventriculus and the pylorus. It is the region of the midgut where the ampullae of the Malpighian tubules open. This region in *I. limbata* is regarded as a part of the midgut since it is not lined with chitinous intima, the columnar epithelial cells are like the columnar midgut cells and very few regenerative cells (RgC) are also present. This region has been variously named and considered as a part of the hindgut by many authors, even though chitinous intima is not present in this region. It has been termed intestine (Khanna, 1964), ileum (Bhaskaran *et al.*, 1969; Mall, 1979; Singh and Sharma, 1987) or pylorus (Kurup, 1964; Muraleedharan, 1983). Snodgrass (1935) has pointed out that the ileum in gymnocerates should be regarded as a segment of the mid-intestine and not to be homologized with the ileum of other insects which is considered as a part of the hind intestine. Rastogi (1962) is of opinion that ileum of phytophagus bugs is truely an endodermal part or at least midgut continuation and the ileum of predaceous bugs is truly a proctodaeal region since there is a chitinous lining in it. The cells of the ampulla (Fig. 14) where the Malpighian tubules open are similar to cells of the fifth ventriculus. As in *I. limbata* the Malpighian tubules open into the extreme end of the midgut in *Rhodnius prolixus* (Wigglesworth, 1931) and *Laccotrephes robustus* (Pakrutty and Mohamed, 1989). In*I. limbata* the fifth ventriculus which is a transitional region does not seem to have any role in digestion and absorption as reported in bees (Serrao and Cruz-Landim, 1996).

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(Received in July 1999; revised and accepted in August 1999)

ENTOMON **24(4):** 331–337 (1999)
Article No. ent.24405

Description of Two New Species of Jumping Spiders (Araneae: Salticidae) of the Genera *Phidippus* Koch and *Plexippus* Koch from Bangladesh

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ABSTRACT: Three species of jumping spiders of the genera *Phidippus* Koch and *Plexippus* Koch are recorded from Bangladesh. Of these, two species namely- *Phidippus majumderi* n.sp. and *Plexippus zabkai* n.sp. are described as new to science. Another one *Plexippus paykulli* (Audouin) is a new record from the country.

KEYWORDS: Jumping spider, New record, *Phidippus*, *Plexippus*, Araneae, Salticidae, Bangladesh.

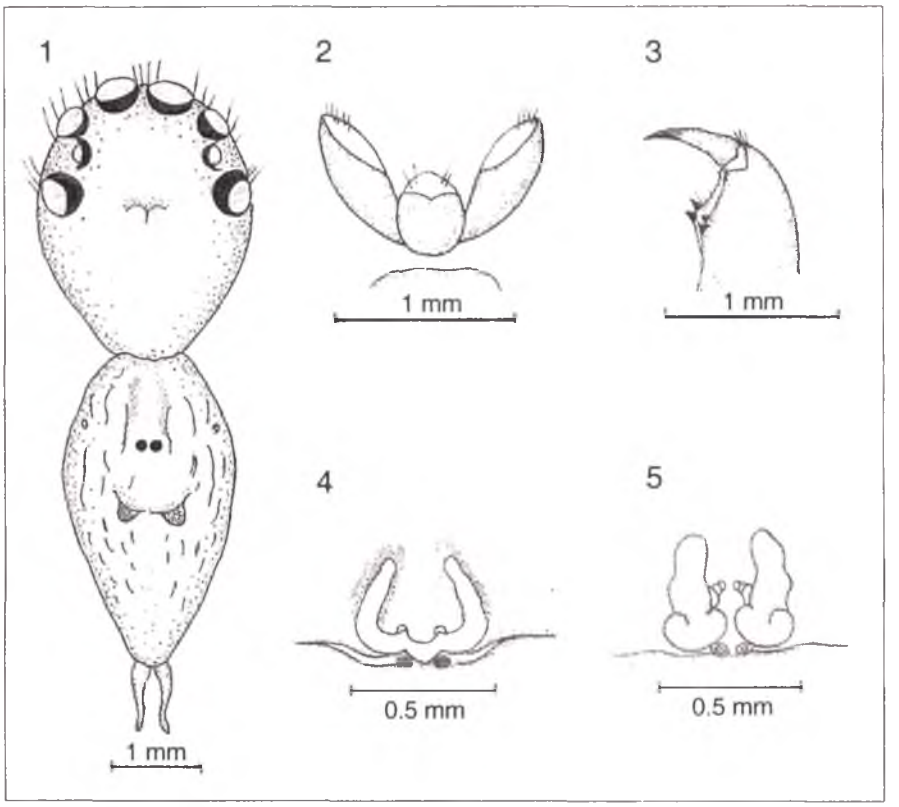
INTRODUCTION

Our earlier report included three species of jumping spiders of Bangladesh of the genera *Plexippus* Koch and *Marpissa* Koch (Biswas and Raychaudhuri, 1997; Biswas and Begum, 1997). Earlier Biswas (1984, 1987), Tikader (1974, 1977), Tikader and Biswas (1981), Biswas and Biswas (1984, 1992) and Proszynski (1992) described different species of salticid spiders from India. Besides these, Peckham and Peckham (1909), Proszynski (1990), Zabka (1985) and Yaginuma (1986) reported many salticids from different parts of the world.

In Bangladesh, there is no taxonomic description of the present genera except a few report made by (Biswas, 1995), Chowdhury and Nagari (1981), Chowdhury and Pal (1984), Biswas *et al.* (1993), Okuma *et al.* (1993) and Begum and Biswas (1997). Present paper deals with one species of the genus *Phidippus* Koch and two species belonging to the genus *Plexippus* Koch. Of these, *Phidippus majumderi* and *Plexippus zabkai* are described as new to science and *P paykulli* Audouin is a new record from Khulna, Bangladesh (marked * in the text).

Phidippus majumderi n.sp. (Figs 1–5)

Female: Cephalothorax and legs yellowish brown; abdomen pale-yellow; Total body length 6.00 mm. Carapace 3.00 mm long, 2.30 mm wide; abdomen 3.00 mm long, 2.00 mm wide. Legs as in table-1.



FIGURES.1–5: 1: Whole body (Dorsal view). 2: Chelicerae. 3: Maxillae and labium and part of sternum. 4: Epigynum. 5: Internal genitalia.

Cephalothorax: Longer then wide; oval in shape, clothed with fine pubescence provided with distinct fovea at the middle of carapace. Eyes pearly-white, bases of which encircled by blackish patches. Anterior row of eyes recurved; median longer than the laterals and close to each other than the laterals. The second row of eyes smallest of all the eyes, the third or posterior row of eyes are the largest of all (Fig. 1). Chelicerae brownish, not so strong, longer than wide; inner and outer margins provided with similar tooth (Fig. 3). Maxillae and labium longer than wide, pale brown, anteriorly whitish, narrowed and scopulate (Fig. 2). Sternum longer than wide, pointed posteriorly. Legs comperatively strong; tibiae and metatarsi of all legs provided with black spines; second pair more robust and longer. Leg formula 2134 and the measurements (in mm):

Abdomen longer than wide, cylindrical, narrowed in both ends and covered with fine pubescence. A pair of dorsal black patches and sigillae present on the dorsum. Epigyne bifurcated anteriorly with copulatory openings (Fig. 4). Internal genitalia with dumble shaped spermathecae (Fig. 5).

TABLE 1. Length of legs of female (♀) holotype of *phidippus majumderi* n.sp. (in mm)

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.1	0.3	0.8	0.8	0.4	3.4
II	1.3	0.4	1.0	1.0	0.5	4.2
III	1.0	0.4	0.8	0.8	0.3	3.3
IV	1.0	0.4	0.7	0.7	0.3	2.1

Male: Unknown.

Holotype: ♀, Mongla port, Khulna, 20.XI.1994, Coll. V. Biswas and M. C. Rudra.

Paratype: 1♀, Otherwise data same as for the holotype.

Distribution: Bangladesh: Khulna (Type locality).

Etymology: The species is named after Dr. S. C. Majumder, Scientist, Zoological Survey of India, Calcutta, for his great contribution in identification of the species.

Remarks: The species *Phidippus majumderi* n.sp. resembles *S. pateli* Tikader (Tikader, 1974) in appearance but differs from that in having the following characters:

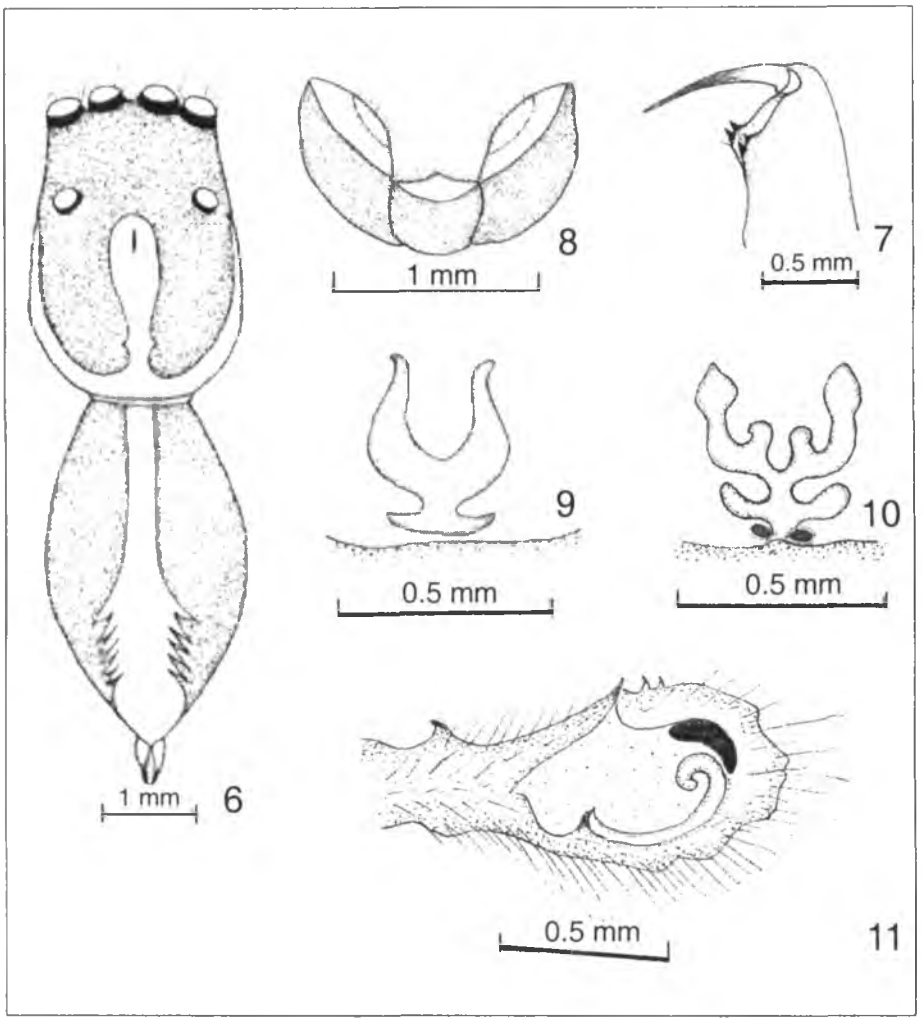
(1) Maxillae anteriorly pointed and whitish in colour.
(2) Labium longer than wide, elongated in form.
(3) Chelicerae broad, not so strong and provided with slightly curved fang; inner and outer margins with two large and two small teeth (Fig. 2).
(4) Different and distinct epigyne and internal genitalia.

Therefore, the species is described as new to science.

Plexippus Zabkai n.sp. (Figs 6–11)

Female: Cephalothorax and legs yellowish brown; abdomen pale-brown. Total body length 6.50 mm. Carapace 3.00 mm long, 2.20 mm wide; abdomen 3.50 mm long, 2.00 mm wide. Legs as in Table 2.

Cephalothorax: Longer than wide, rectangular, cephalic region flat, provided with distinct fovea at the middle, clothed with fine pubescence. Eyes transparent, bases of which encircled by black patches (Fig. 6). Anterior row of eyes equal in size, slightly recurved, distributed in equal distance from each other. Second pair of eyes very minute; posterior row slightly smaller than the anterior row; numerous hairs and spines arise from the base of the anterior row (Fig. 6). Chelicerae strong, reddish brown, inner and outer margins with the similar teeth (Fig. 7). Maxillae pale yellow, longer than wide, both ends narrowed, whitish in the middle and slightly scopulate. Labium



FIGURES.6–11. 6: Whole body (Dorsal view). 7: Chelicerae. 8: Maxillae and labium 9: Epigynum. 10: Internal genitalia. 11: Male palp.

slightly longer than wide, anteriorly whitish and slightly scopulate (Fig. 8). Sternum longer than wide. Legs strong and stout, clothed with hairs and spines. Leg formula 4312 and the measurements (in mm):

Abdomen: Longer than wide, nearly oval, covered with fine pubescence. Dorsum provided with a mid-longitudinal band extends from the base of the carapace to the base of the spinnerets. Ventrally uniformly coloured. Epigyne as a rounded plate,

TABLE 2. Length of legs of female (♀) holotype of *Plexippus zabkai* n. sp. (in mm)

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.0	0.3	0.6	0.7	0.4	3.0
II	0.8	0.3	0.5	0.6	0.3	2.5
III	1.2	0.4	0.7	0.8	0.4	3.5
IV	1.3	0.4	0.8	0.9	0.6	4.0

bifurcate anteriorly with copulatory opening (Fig. 9). Internal genitalia with coiled copulatory sac divisible into two parts (Fig. 10).

Male: Smaller than the female, same in colour. Total length 5.60 mm. Carapace 3.00 mm long, 2.10 mm wide; abdomen 2.60 mm long, 2.00 mm wide. Body longer than wide. Tibiae of male palp provided with retrolateral apophysis; tegulum flat and convex, provided with tegular apophysis; embolus coiled (Fig. 11).

Holotype: ♀, Morelganj, Dist. Bagerhat, 19. IV. 1993, Coll. V. Biswas.

Paratype: 2♀, Otherwise data same as for the holotype.

Allotype: 1♂, Otherwise data same as for the holotype.

Distribution: Bangladesh; Bagerhat (Type locality).

Etymology: The species is named after Dr. Marek Zabka, a renowned polish Arachnologist, in recognition of his kind help in this work.

Remarks: The species *Plexippus zabkai* n.sp. resembles *P. paykulli* (Audouin) (Tikader and Biswas, 1981) in general appearance but it differs from that in the following characters:

(1) Cephalothorax provided with prominent fovea; antero-median and anterolateral eyes similar in shape whereas in *P. paykulli* (Audouin) cephalothorax with very small fovea and anteromedian eyes two times larger than the anterolaterals.

(2) Labium rectangular; maxillae narrower in both ends, inner side provided with whitish patches. In *P. paykulli* (Aud.) labium roundish; maxillae wider anteriorly and without white patches at the inner side.

(3) Epigyne and male palp structurally different.

The species, is therefore, described as new to science.

***Plexippus paykulli** (Audouin)

1825. *Attus paykulli* Audouin, Descr. Egypt., 22 : 172.

1998. *Plexippus paykulli*: Biswas & Raychaudhuri, Rec. Zool. Surv. India, 96(1–4): 167.

Material examined: 2♀, Bagerhat, 12. VII. 1993, Coll. V. Biswas; 1♀, Dhaka, 12. Vi. 1994, Coll. V. Biswas; 2♀, Faridpur, 19. XI. 1994, Coll. V. Biswas; 2♀, Jhenidah, 12.VIII.1993, Coll. V. Biswas; 1♀, 1♂, Khulna, 18.X.1994, Coll. V. Biswas; 2♀, Kustia, 15. V. 1993, Coll. V. Biswas; 1♀, Sylhet, 18. V. 1994, Coll. V. Biswas.

Distribution: Bangladesh: Bagerhat, Jhenidah, Khulna, Kustia, Dhaka, Faridpur, Sylhet; America; Africa; Burma; China; India; Japan; Srilanka; Europe.

The identity of the species was confirmed at the Zoological Survey of India, Calcutta. The types are preserved at present in the Department of Zoology, Government P. C. College, Bagerhat and will be deposited with the Museum of the Department of Zoology, University of Dhaka, Bangladesh, in due course of time.

ACKNOWLEDGEMENTS

The author is grateful to Dr. S. C. Majumder, Scientist-SD, Sunderban Field Research Station, Zoological Survey of India, Canning, West Bengal, India, for confirming the identity of the species and the Head, Department of Zoology, Government P. C. College, Bagerhat, Bangladesh, for providing the laboratory facilities.

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(Received in January 1999; revised and accepted in July 1999)

ENTOMON 24(4): 339–343 (1999)
Article No. ent.24406

Development, Life Table and Intrinsic Rate of Natural Increase of Three Morphs of *Rhynocoris marginatus* Fab. (Heteroptera: Reduviidae) on Cotton Leaf Worm *Spodoptera litura* Fab. (Lepidoptera: Noctuidae).

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ABSTRACT: The development and life table studies on three morphs of *Rhynocoris marginatus* Fab. (niger, nigrosanguineous and sanguineous) an important reduviid predator of the cotton leaf worm *Spodoptera litura* Fab. were studied in the laboratory. Maximum fecundity (172.20 ± 27.99) and longevity (73.94 ± 13.92) were observed in niger morphs followed by nigrosanguineous and sanguineous morphs. The net reproductive rates were 56.50, 50.90 and 37.20 for niger, nigrosanguineous and sanguineous morphs, respectively. The weekly multiplication rate was highest on niger morphs (1.342) followed by nigrosanguineous (1.310) and sanguineous morphs (1.278).

KEYWORDS: *Spodoptera litura*, three morphs of *Rhynocoris marginatus*, development, life table.

INTRODUCTION

Life table is the most useful numerical aid in studying population biology (Southwood, 1978) enabling determination of age distribution and mortality rate in natural population. Such studies facilitate assessment of values of various ingredients of environment which are responsible for maintanence of population in nature. Reduviidae is the largest family of predaceous land Heteroptera with considerable potential to act as biological control agents. *Rhynocoris marginatus* Fab. is a reduviid oraciously predates on various economically important insect pests (Ambrose, 1996, 1999).

R. marginatus has very striking variation among individuals occuring in the same microhabitat that would compel a museum taxonomist to place them comfortably in different species altogether. It exists in three different morphs (1) with black connexivum (niger), (2) with red connexivum (sanguineous) and (3) with black and red banded connexivum (nigrosanguineous) (Ambrose and Livingstone, 1988). The present study was undertaken to find out whether there is any variation in the biology and life table characteristics of these morphs, which is essential to conserve and augment the better adopted morph for their utilization in biocontrol programme.

MATERIALS AND METHODS

Adult and nymphal instars of all the three morphs of *R. marginatus* were collected from Sivanthipatti agroecosystem in Tirunelveli district, Tamilnadu (altitude 125.33 ± 2.87; lattitude 77° 21E and 8° 31N). They were reared in the laboratory (temp. 30 ± 2 °C, humidity 80-85%; photoperiod 11–13 h) separately in plastic containers (8 × 6 × 4 cm) on *Spodoptera litura* (Fab.). A cohort consisting of 100 eggs from each morph was used to construct life tables. Eggs were collected and allowed to hatch in small plastic containers with moistened cotton swabs for maintaining optimum humidity (85%). The cotton swabs were changed periodically to prevent fungal attack. After hatching, all the nymphs were reared individually in plastic containers and *S. litura* was provided as the prey. Observations were made on hatching, completion of nymphal development, successful adult emergence, fecundity and age specific mortality in respective stages. After the emergence of adult, the life table was constructed separately for the different morphs. The life tables were constructed by determining and recording the each age interval, the survival rate (l_x) and the mean number of female progeny per female (m_x) still alive at such age intervals. The intrinsic rates of increase of population in different morphs were calculated. The studies were made by using Birch's (1948) formula elaborated by Watson (1964) and Southwood (1978).

In life table statistics the intrinsic rate of increase was determined by using the equation $\sum e^{-r_m x} l_x m_x - 1$.

Where e is the base of natural logarithms, x is the age of the individuals in days, l_x is the number of individuals alive at age x as the proportional of 1, and m_x is the number of female offsprings produced per female in the age interval x. The sum of products $l_x m_x$ is the net reproductive rate (R_o). The rate of multiplication of population for each generation was measured in terms of females produced per generation. The precise value of cohort generation was calculated as follows.

$$T_c = \frac{\sum l_x m_x}{R_o}$$

The arbitrary value of innate capcity for increase r_c was calculated from the equation

$$r_c = \frac{\log_e R_o}{T_c}$$

This is an appropriate r_m value. The values of negative exponent of $e^{-r_m x}$ ascertained from this experiment often lay outside the range. For this reason both sides of the equation were multiplied by a factor of $\sum e^{7-r_m x} l_x m_x - 1096.6$ (Birch, 1948; Watson, 1964). The two values of $\sum e^{7-r_m x} l_x m_x$ were then plotted on the horizontal axis against their respective arbitrary r_m on the vertical axis. Two points were then joined to give a line which was intersected by a vertical line drawn from the desired value of $e^{7-r_m x} l_x m_x$ (1096.6).

The point of intersection gives the value of r_m accurate to three decimal places. The

TABLE 1. Biology of three morphs of *R. marginatus* on *S. litura* ($\bar{x} \pm$ SD)

Parameters	Morphs		
	Niger	Nigrosanguineous	Sanguineous
Incubation period	9.60 ± 0.50^a	8.15 ± 0.37^b	9.50 ± 0.61^a
Total developmental period	92.29 ± 2.52^a	75.38 ± 1.09^b	99.47 ± 1.64^a
Longevity	73.94 ± 13.92^a	64.06 ± 13.26^b	53.73 ± 9.54^c
Pre-oviposition period	31.25 ± 2.48^a	26.33 ± 2.49^b	24.28 ± 3.50^c
Fecundity	172.20 ± 27.99^a	149.44 ± 24.55^b	123.56 ± 17.56^c

Means followed by the sample alphabet in a row are not statistically singificant at 5% ($P > 0.05$) by Tukey test.

precise generation time (T) was then calculated from equation

$$T = \frac{\log_e R_o}{r_m}$$

The finite rate of increase (λ) was calculated as e^r_m. The weekly multiplication of predator population was calculated as $(e^r_m)^7$. The doubling time was calculated as $\log 2 / \log \lambda$.

RESULTS AND DISCUSSION

The incubation period of the niger morph of *R. marginatus* was 9.60 ± 0.50 days and it was reduced to 8.15 ± 0.37 and 9.50 ± 0.61 days in nigrosanguineous and sanguineous morphs, respectively (Table 1). Duration of the post embryonic development, fecundity, longevity and number of prey consumed during their life time play a paramount importance in assessing the predatory efficiency of an individual (Ananthakrishnan, 1996). The longevity of niger morph was more (73.94±13.93 days) than nigrosanguineous (64.06 ± 13.26 days) and sanguineous (53.73 ± 9.54 days) morphs. The fecundity of niger morph was also maximum (123.56 ± 27.99) than nigrosanguineous (149.44 ± 24.55) and sanguineous (123.56 ± 17.56) morphs. The shorter developmental period and highest longevity and fecundity observed in niger morph might be due to the adaptation gained by this morph in their ecosystem.

Table 2 provides life table parameters of three morphs of *R. marginatus*. The population growth statistics of the three morphs of *R. marginatus* revealed that the net reproductive rate (56.50, 50.90 and 37.20 for niger, nigrosanguineous and sanguineous morphs, respectively) was lesser than the gross reproductive rate (86.00, 75.00 and 62.00 for niger, nigrosanguineous and sanguineous morphs, respectively) due to sharp decline in the survivorship value of the parent females. The mean length of generation was shorter on niger morph followed by nigrosanguineous and sanguineous morphs. Consequent to the decrease in r_m and extension of developmental period, the population doubling time of nigrosanguineous and sanguineous morphs increased than the niger morph. The weekly multiplication rate was also higher on niger morph (1.342) than nigrosanguineous (1.310) and sanguineous morphs (1.278).

TABLE 2. Life table statistics of three morphs of *R. marginatus* on *S. litura*

Parameters	Morphs		
	Niger	Nigrosanguineous	Sanguineous
Gross reproductive rate (GRR)	86.00	75.00	62.00
Net reproductive rate (R_0)	56.50	50.90	37.20
Mean length of generation (T_c)	127.78	124.84	107.43
Innate capacity for increase in numbers (r_c)	0.04	0.03	0.03
Corrected r_m	0.042	0.038	0.035
Corected generation time (T)	106.17	103.32	93.57
Finite rate of increase	1.043	1.039	1.036
Weekly multiplication rate (WMR)	1.342	1.310	1.278
Doubling time (DT)	16.44	18.12	19.60
Annual rate of increase	4.5×10^6	1.0×10^6	0.35×10^6
Hypothetical female in F2 generation	3192.25	2590.81	1383.84

The variation in the life table statistics exhibited by the morphs of *R. marginatus* revealed the intraspecific variations of an ecotype. Mayr (1963) briefly defined polymorphism as variability within a population. Ford (1937) explained polymorphism as the " occurrence together in the same habitat of the same species in such a proportion that the rarest of them can't be maintianed by recurrent mutation". Breeding experiments between morphs of a particular ecotype of Ambrose and Livingstone (1988) revealed that such intraspecific variations were not strictly genetic. They further reported that the level of population of different morphs both in the field and in laboratory suggested that the seggregation phenomenon did not occur in the Mendelian fashion. However, the adaptive siginificance of a particular morph in a given ecosystem can not be ruled out as evidenced by niger with its higher insecticide resistance (George and Ambrose, 1996). The present study established the variation in the life table characteristics of different morphs of *R. marginatus* and suggested that the effective niger morphs could be selected for the biocontrol programme. Anyhow, further investigations are required to understand this phenomenon of polymorphism better.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Rev. Fr. Antony A. Pappuraj s.j, Principal, St. Xavier's College, Palayankottai for the facilities, Dr. Dunston P. Ambrose, Director, Entomology Research Unit, St. Xavier's College, my supervisor for the continued encouragement and DST New Delhi for financial assistance (Young Scientist Scheme HR/OY/Z-07/97).

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(Received in March 1999; revised and accepted in June 1999)

ENTOMON 24(4): 345–352 (1999)
Article No. ent.24407

Insecticide Resistance and its Mechanism in *Culex quinquefasciatus* Mosquitoes from Mumbai city, Maharashtra State, India

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ABSTRACT: Insecticide susceptibility studies were carried out on six strains of *Cx. quinquefasciatus* mosquitoes collected from the central and peripheral areas of Mumbai city. Bioassays showed that all the strains were resistant to DDT, Malathion and Porpoxur. Insecticide resistance status was the same in both the larval and adult stages. Bio-chemical analysis of these populations showed that there was an increase in the activity of glutathione s-transferase enzyme in DDT resistant strains as compared to the susceptible strain. Similarly, all the organophosphate and carbamate insecticide resistant strains showed higher esterase activity than the colony strain. None of the strains showed resistance to Deltamethrin. It was very interesting to note that Dahisar (suburban) and Santacruz (airport area) strains showed multiple resistance mechanisms to organophosphate and carbamate insecticides.

KEYWORDS: Mumbai city, Insecticides, Susceptibility, Multiple resistance, Resistance mechanism.

INTRODUCTION

Filariasis is one of the six important tropical diseases. Globally about 80 million people are infected with *Wuchereria bancrofti*, the filarial worm. Of these about 30 million chronic cases manifest as typical elephantiasis (WHO, 1995). This disease is highly prevalent in the developing countries like India where *Culex quinquefasciatus* mosquito is the only vector. The prevalence of this vector is steadily increasing in the urban situations due to continuous developmental projects in almost all the cities causing more and more mosquitogenic conditions and spread of mosquito-borne diseases in newer areas. Mumbai, which is considered as the commercial capital of this country, is expanding in geometric proportions leading to large-scale migrations of people from rural areas to the city. This has indirectly resulted in the development of slums in almost every corner of the city. These slums without proper sanitation facilities lead to the accumulation of toxic, polluted water and open drainages. These

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conditions have provided numerous breeding habitats for this mosquito species. *Cx. quinquefasciatus* is well adapted to such type of habitats.

Mosquito control programs in such situation have failed due to various reasons. Development of insecticide resistance in the mosquitoes is one of the important reasons. Despite the urban development the suburban areas show rural characteristics in the peripheral regions, where agricultural practices are still continued and a lot of agrochemicals are in use. This has also helped in the development of insecticide resistance in the vector species.

The present study is an attempt to understand the dynamics of insecticide resistance in this mosquito species in different areas of Mumbai city. Initial studies on *Cx. quinquefasciatus* showed a high degree of resistance to different insecticides in different areas, hence further work was carried out to understand the resistance mechanisms involved.

MATERIAL AND METHODS

Mosquitoes

Cx. quinquefasciatus adults were collected from different sites in and around Mumbai city. Dusk collections in different peripheral areas were done in the cattle sheds while in the central city areas indoor house collections were carried out. Adult mosquitoes were collected with the help of aspirators. These were transported to the laboratory having insectary conditions of 28 ± 2 °C, 80 ± 10RH, and 13 : 11 LD. Adults were confined in 30 × 30 × 30 cm screened cages and fed on 10% glucose solution in moistened cotton pads, till the fed females laid egg rafts. Unfed females were fed on white leghorn and maintained similarly till they laid egg rafts. Larval collections were also done in both central and peripheral areas of Mumbai city. Field collected larvae were reared in enamel pans containing tap water and were fed on a mixture of yeast powder and dog biscuit (1 : 1).

Larval bioassay

This was carried out with ethanol concentrations of DDT, Malathion, Propoxur & Deltamethrin prepared from the technical grade of insecticide. Technical grade Deltamethrin was procured from Roussel India, Propoxur was procured from Bayer India, Malathion was procured from Fiscon India Ltd while DDT was procured from Sigma Chemical Co., USA. The WHO method was followed for larval bioassay (WHO, 1981b). Prohibit analysis (Finney, 1971) was applied to the mortalities obtained for the different concentrations of the larvicides to calculate LC50 for different mosquito strains.

Adult bioassay

Field collected 20–25 adults female mosquitoes were exposed to the insecticide impregnated papers. Insecticide impregnated papers were prepared locally by the method of Busvine and Nash (1953). Tests were performed as per the protocol outlined

by WHO (1981a). The dosages were, DDT 4%, Deltamethrin 0.025%, Porpoxur 0.1% and Malathion 5%. After exposure, the mosquitoes were maintained in the insectary at 28±2 °C and 80–90% RH. Cotton pads soaked in 10% glucose solution were provided during the recovery period of 24 hours. The percent mortality count was done 24 hours after exposure.

Enzyme assays

Assays were performed on female mosquitoes. Adults emerged from the field collected larvae were stored at −70 °C until assayed. Mosquitoes were homogenised in distilled water with the help of plastic pestle (Kontes) in microfuge tubes and centrifuged at $10,000 \times g$ for 10 minutes. The homogenates were centrifuged at 10,000 rpm for 4–5 min at 4 °C in eppendorf tubes, and the supernatant transferred to clean microtitre plates. The methods followed for esterase (Est A & B), Acetylcholinesterase (AChE) and Glutathione s-transferase (GST) were as mentioned below;

Protein assays

The protein content was estimated in 40 μl of supernatant fluid from each individual homogenate by the method described by Lowry *et al.* (1951). A reference standard protein curve was prepared using Bovine Serum Albumin fraction 5.

Esterase (Est)

Assays were performed by the method of Hemingway *et al.* (1986) with 2 × 20 μl aliquots of supernatant from each individual, using 1- and 2-naphthyl acetate as substrates. After incubation for 10 min at 37 °C, the reaction was stopped by the addition of stain, (fast blue RR in 5% sodium dodecyl sulphate solution) and the end-point absorbency measured at 490 nm in UV max microplate reader. Results were converted to absolute units by analysis against standard curves for 1- and 2-naphthol and adjustment for protein concentration.

Acetylcholinesterase (AChE)

Normal and Propoxur-inhibited AChE activity was determined as described by ffrech-Constant and Bonning (1989), in 2 × 30 μl aliquots of supernatant from each individual mosquito. In these assays, the final concentration of Propoxur was 0.2 mM; higher concentrations totally inhibited AChE activity in *Aedes aegypti* (Mourya *et al.*, 1993b).

Glutathione-transferase (GST)

Its activity was determined by the method of Habig *et al.* (1974). To 25 μl of the supernatant from each homogenate was added 80 μl of a mixture of freshly prepared reduced glutathione (0.1 M) in phosphate buffer (pH 6.5) and 0.48 μl of 3,4,-chloro-dinitrobenzene (CDNB) in methanol (15 nMol). Rate reactions were measured for 5 min at 37 °C. Enzyme activity was calculated with the extinction co-efficient for CDNB $I > E = 9.5$/mM/cm and the protein concentration of the sample. Enzyme activity was expressed as activity/min/mg protein.

TABLE 1. Susceptibility of different strains of *Culex quinquefasciatus* larvae to insecticides.

Strains	LC50 (mg/L)			
	Malathion	Propoxur	Deltamethrin	DDT
COLONY	0.0379 (0.025–0.045)*	0.0517 (0.03–0.06)	0.0015 (0.0008–0.002)	0.131 (0.045–0.21)
DHAHISAR	0.7889 (0.61–0.84)	0.6834 (0.45–0.82)	0.0017 (0.001–0.0023)	0.449 (0.2–0.65)
DADAR	0.3825 (0.24–0.45)	0.3406 (0.21–0.46)	0.0015 (0.0006–0.0026)	0.578 (0.32–0.71)
SANTACRUZE	0.8109 (0.67–0.96)	0.7716 (0.63–0.89)	0.0018 (0.001–0.0022)	0.457 (0.28–0.62)
BANDRA	0.2391 (0.11–0.32)	0.4086 (0.28–0.61)	0.0019 (0.001–0.003)	0.567 (0.38–0.81)
BORIVLI	0.6482 (0.44–0.84)	0.6651 (0.55–0.87)	0.0016 (0.001–0.0022)	0.675 (0.42–0.91)

* = Fiducial limits ($n = 80$)

Polyacrylamide gel electrophoresis (PAGE)

The method followed for qualitative assays for esterase enzyme is largely same as described by Chakraborti *et al.* (1993).

RESULTS

Insecticide bioassay performed on different strains of *Cx. quinquefasciatus* mosquito showed that all the five strains were resistant to DDT as compared to the susceptible colony strain. The adult bioassays showed a varying degree of susceptibility to DDT, Malathion and Propoxur while all the strains were equally susceptible at both the larval and adult stages to Deltamethrin (Tables 1 & 2). PAGE performed for Est enzyme on the homogenates of larvae & adults showed an increased activity as compared to the control strain. However, it was highest in Dahisar & Santacruz strains (Figure). When quantitative assays were performed on these strains showed that the frequency of mosquitoes with high esterase activity was more in Dahisar & Santacruz as compared to the colony and other three strains (Table 3). Biochemical assays performed on these strains showed about 2-fold increase in the GST enzyme activity in both the larvae and adults as compared to the susceptible colony strain. Similarly all the strains showed resistance to Malathion & Propoxur, however it was higher in the Dahisar & Santacruz strains. AChE assays conducted on these strains using acetylthiocholine iodide as substrate showed that there were no noticeable differences in the enzyme activity among these strains. However, percent inhibition of AChE enzyme activity by Propoxur was low in the case of larvae and adults of Dahisar and Santacruz strains (Table 4), thus suggesting possible involvement of altered AChE in these two strains.

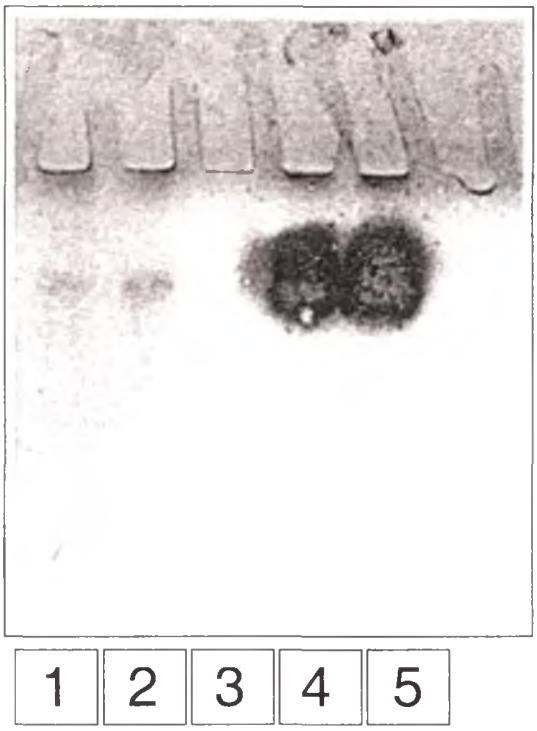

FIGURE 1. Expression of esterase enzyme in the adults of different strains of *Culex quinquefasciatus*: [Lane 1] Control strain; [Lane 2] Control strain; [Lane 3] Empty; [Lane 4] Dahisar strain; [Lane 5] Santacruz strain.

TABLE 2. Susceptibility of different strains of *Culex quinquefasciatus* adults to insecticides

Strains	Percent mortality			
	Malathion	Propoxur	Deltamethrin	DDT
COLONY	98	98	100	99
DHAHISAR	25	39	100	22
DADAR	30	47	98	21
SANTACRUZE	37	25	89	31
BANDRA	25	53	93	32
BORIVLI	28	25	95	27

DISCUSSION

The insecticide resistance mechanisms in all the studied strains of *Cx. quinquefasciatus* from Mumbai showed an interesting spectrum. Earlier studies in India with

TABLE 3. Frequency analysis of mosquitoes showing varying activity of esterase enzyme in different strains. (nMol activity/min/mg protein)

Range	COLONY	DHAHISAR	DADAR	SANTACRUZE	BANDRA	BORIVLI
			Est-A(Larvae)			
10–20	100	8	58	33	50	58
30–100	0	75	42	50	50	33
120–300	0	17	0	17	0	8
Total	100	100	100	100	100	100
			Est-A(Adults)			
10–20	100	8	35	0	53	28
30–100	0	65	55	33	33	50
120–300	0	27	10	67	14	5
Total	100	100	100	100	100	83
			Est-B(Larvae)			
10–20	100	0	42	25	55	50
30–100	0	75	58	50	40	42
120–300	0	25	0	25	5	8
Total	100	100	100	100	100	100
			Est-B(Adults)			
10–20	100	12	32	12	63	38
30–100	0	61	62	70	34	58
120–300	0	27	6	18	3	4
Total	100	100	100	100	100	100

TABLE 4. Enzyme activity in five stains of *Cx. quinquefasciatus* mosquitoes. (Activity/min/mg protein)

Strains	GST	SD	AChE	SD	% AChEinhibition
			LARVAE		
COLONY	5.2	1.7	155.5	65.7	100.0
DHAHISAR	11.2	3.2	202.9	71.5	53.0
DADAR	12.1	3.2	187.3	47.1	92.0
SANTACRUZE	14.7	3.0	164.5	65.2	48.0
BANDRA	14.3	5.0	181.3	60.2	80.0
BORIVLI	13.4	2.1	169.7	71.7	97.0
			ADULTS		
COLONY	9.5	2.5	496.4	73.2	99.0
DHAHISAR	16.8	3.2	704.7	89.7	46.0
DADAR	18.5	4.0	536.4	71.8	89.0
SANTACRUZE	19.6	3.7	696.1	81.9	45.0
BANDRA	21.8	4.8	546.0	90.5	87.0
BORIVLI	23.8	4.6	527.2	88.3	94.0

reference to insecticide resistance in mosquitoes showed prevalence of widespread resistance to DDT in *Ae. aegypti* which was found to be incurred due to increased GST activity (Mourya *et al.*, 1993a). It seems that in these strains also the DDT resistance is due to GST based mechanism. It appears that even after the ban on DDT, chlorinated hydrocarbons are still being used.

The *Cx. quinquefasciatus* populations from two localities *viz.* Santacruz (urban) and Dahisar (suburban) showed higher Malathion and Propoxur resistance. It appears that the higher level of resistance is due to an increased activity of Est enzyme and altered AChE. The association of increased Est activity with organophosphate and carbamate insecticide resistance in *Cx. quinquefasciatus* seems to be a global phenomenon (Small *et al.*, 1998). Recently it has been shown that the increase Est activity can also cause cross resistance to Deltamethrin (Brogdon and McAllister, 1998). However, it was interesting to note that none of the strains showed resistance to this insecticide.

Data suggest that resistance in the mosquitoes from the suburb area like Dahisar may be due to heavy use of pesticides. Similarly, the airport area should also be under heavy coverage of insecticides due to international airport regulation. This may be the cause of high & multiple resistance in the mosquitoes from this region. This data also suggest that the involvement of multiple mechanisms of resistance to these insecticides could be one of the important reasons for the chemical control of this species. The development of resistance in this species from two different areas could be due to several ecological and man made factors. It appears that for an effective filariasis control there is a need to pose strict legislation and environmental management strategy.

ACKNOWLEDGEMENTS

The senior author thanks Mr. N. S. Rao & Mr. Anil Rao, chairman & Managing Director, Pest Control (India) Ltd. Mumbai, for their encouragement & help in the present studies. The financial support and laboratory facilities provided by the Haffkine Institute for Training Research & Testing, Mumbai is duly acknowledged.

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(Received in May 1999; revised and accepted in August 1999)

ENTOMON 24(4): 353–358 (1999)
Article No. ent.24408

Effect of Different Host Diets on the Grasshopper, *Diabolocatantops pinguis* (Walker)

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ABSTRACT: Effect of four different host diets on *Diabolocatantops pinguis* was studied. One-way ANOVA was done to establish the difference in the effect of the hosts on developmental patterns, fecundity, mortality and other such life-history parameters. Among the four economically important hosts tested, *Arachis hypogaea* and *Phaseolus aureus* were significantly better hosts for this acridid species than *Helianthus annuus* or *Sorghum vulgare*. Mortality was very low, and was observed only in the initial stages of development.

KEYWORDS: *D. pinguis*, biology, host plants, physico-chemical characters, acridids.

INTRODUCTION

Investigations on intrinsic and extrinsic factors that influence growth and development of insects have always attracted the attention of entomologists. Nutrition is an important extrinsic factor in all insect species (Dadd, 1985). Polyphagous grasshoppers which feed on a variety of hosts were observed to grow faster than mono- and oligophagous species which feed on one or few host species (Lee, 1990). Many workers (Chapman and Joern, 1990; Suresh and Muralirangan, 1996) have reported the influences of host diets on the biology of acridids. Deficiency in carbohydrate and protein supplies during nymphal development result in slower growth rates (Dadd, 1985).

D. pinguis is a minor pest (COPR, 1982) infesting several economically important crops. It is widely distributed in grasslands, agroecosystems and forests and was observed to feed on at least 20 species of agricultural crops (Vedham, 1994). Here, an attempt was made to study the influence of different host plants on the biology of *D. pinguis*, particularly on aspects like postembryonic development, fecundity, mortality and optimal growth rate. This study was taken up at the Department of Zoology, Guru Nanak College, Chennai.

Test insects were taken from host specific stock cultures, reared and maintained in wooden cages in the laboratory, under natural photoperiod of 11.13L : 12.47D to

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12.47L : 11.13D and temperature of 21.0 °C to 36 °C. Mature leaves of host plants were kept in Knop's solution to prevent wilting, when provided as food. Four host species *viz.*, *A. hypogaea, P. aureus, H. annuus* and *S. vulgare* were selected for the experiment based on the quantitative consumption data (Vedham, 1994) and the occurrence of *D. pinguis* on these host plants in agroecosystems. Freshly hatched nymphs were reared on each of the four host plants until adult stage to estimate the postembryonic developmental period and the adult life span. The duration between adult emergence and first mating was recorded by isolating pairs of male and female adults in cages soon after their emergence. Moist soil was provided for oviposition to determine the duration between adult emergence and first oviposition (preoviposition period) and the total number of eggs laid per female. Freshly oviposited eggpods were isolated to determine incubation period and egg hatchability. One way ANOVA was used to analyze the differences in the influence of the host plants on the biology of *D. pinguis*. Physical components like number of trichomes and thickness of leaves and chemical characters like total carbohydrates (Dubois *et al.*, 1958), proteins (Lowry *et al.*, 1951), lipids (Folch *et al.*, 1957), nitrogen (Humphries, 1956) and phenols (Bray and Thrope, 1954) were estimated to analyze their influence on the biology of *D. pinguis*.

D. pinguis has five nymphal instar stages, with female nymphs having longer developmental duration than male nymphs, and, first and fifth instar nymphs has the shortest and the longest developmental periods, respectively, on all four host diets (Table 1).

The developmental duration was similar in those nymphs fed on *A. hypogaea* and *P. aureus*. Significant differences were observed in nymphal durations with respect to all four host diets, except in the first instar stage. The second instar nymphs fed on *A. hypogaea*, *P. aureus* and *H. annuus* developed significantly faster than those fed on *S. vulgare*. The third instar nymphs fed on *H. annuus* developed faster than those fed on other three hosts. The development of the fourth instar nymphs were significantly faster when fed on *A. hypogaea* and *P. aureus* than those fed on *H. annuus* and *S. vulgare*. Among the fifth instar nymphs, those fed on *H. annuus* developed slower than those fed on other three diets. In total, the nymphs developed slower when fed on *S. vulgare* or *H. annuus* than when fed on either of the other two host species. Nymphal mortality occurred only up to third instar stage on all four host plants. Nymphs fed on *S. vulgare* had high mortality during the third instar stage (10.5%), while those fed on *A. hypogaea* and *P. aureus* had negligible nymphal mortality (2.2% and 1.1%, respectively), with the influence of diets on mortality being significantly different (Table 1).

The adults lived longer on *A. hypogaea* and *P. aureus* than on the other two hosts. They had a mean life span of 103.5 days when fed on *A. hypogaea*. It is interesting to note that one of these adults survived for 125 days. The adult longevity was significantly longer in those adults fed with *P. aureus* (98.8 days) than *S. vulgare* (83.2 days) (Table 1). The adult sex ratio was around 1 : 1, on all hosts tested.

TABLE 1. Life history parameters of *D. pinguis* fed on four host diets

Host diets	Nymphal duration*					Adult longevity*	Premating duration*	Preoviposition period*
	I instar	II instar	III instar	IV instar	V instar			
A. hypogaea	7.0 ± 0.9	7.7 ± 1.2	9.7 ± 0.8	7.8 ± 0.8	10.7 ± 0.8	103.5 ± 11.6	9.83 ± 0.8	17.3 ± 2.0
P. aureus	6.7 ± 0.8	8.0 ± 0.6	9.8 ± 0.8	8.3 ± 0.5	10.5 ± 0.5	98.8 ± 6.2	10.3 ± 0.8	17.7 ± 1.9
H. annuus	7.0 ± 0.9	7.5 ± 0.8	8.0 ± 0.9	10.3 ± 1.0	10.2 ± 0.8	88.8 ± 5.7	10.3 ± 1.0	20.7 ± 1.5
S. vulgare	7.3 ± 0.8	9.2 ± 0.8	9.5 ± 0.8	10.2 ± 0.8	10.3 ± 0.5	83.2 ± 7.6	13.8 ± 2.3	24.3 ± 1.6
F value	—	4.3	6.3	15.7	5.3	7.8	10.7	20.7
Level of significance	NS	0.01	0.003	0.0001	0.007	0.001	0.0002	0.0001

Host diets	Fecundity (eggs/♀)	Incubation period*	Hatchability (%)	Nymphal mortality (%)		
				I instar	II instar	III instar
A. hypogaea	230.3 ± 15.9	33.7 ± 3.1	87.6 ± 0.03	10.0 ± 2.8	6.1 ± 2.6	2.2 ± 0.5
P. aureus	218.7 ± 12.3	33.7 ± 5.6	84.7 ± 0.1	11.7 ± 2.0	6.1 ± 2.2	1.1 ± 0.6
H. annuus	204.0 ± 10.8	35.7 ± 2.1	76.9 ± 0.1	15.6 ± 2.7	8.9 ± 3.2	3.9 ± 1.6
S. vulgare	168.7 ± 7.1	38.5 ± 2.7	74.8 ± 0.1	22.2 ± 2.7	16.7 ± 3.3	10.6 ± 2.3
F value	30.2	—	5.4	6.9	3.2	13.5
Level of significance	0.0001	NS	0.006	0.002	0.04	0.0001

* in days
NS - not significant at $p<0.05$
All values are mean ± SD

TABLE 2. Physico-chemical characteristics of the four host species of *D. pinguis*.

	Host species			
	A. hypogaea	*P. aureus*	*H. annuus*	*S. vulgare*
Physical factors				
No. of trichomes ($/\mu^2$)	25.0 ± 1.9	18.3 ± 1.3	47.5 ± 2.9	59.2 ± 2.4
Thickness of leaf (μ)	271.8 ± 3.0	245.2 ± 2.9	328.1 ± 4.2	343.8 ± 5.8
Chemical factors				
Total carbohydrates (mg/g)	17.1 ± 0.3	306.5 ± 7.7	39.7 ± 2.0	290.3 ± 6.8
Total proteins (mg/g)	92.4 ± 7.6	140.3 ± 9.2	73.5 ± 6.8	31.4 ± 2.2
Total lipids (mg/g)	230.2 ± 10.3	76.0 ± 2.6	208.9 ± 12.7	9.3 ± 0.6
Total nitrogen (%)	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.03	0.2 ± 0.02
Total phenols (mg/g)	24.8 ± 1.1	1.0 ± 0.1	12.7 ± 1.1	4.8 ± 0.2

The number of days taken for the first mating after adult emergence was shortest in those fed on *A. hypogaea*, and the longest in those fed on *S. vulgare* (Table 1), and were significantly different. Subsequent mating occurred only after first oviposition and the mating intervals did not vary in all the four cases. All female insects mated three to four times during its lifetime. The adult females reared on *A. hypogaea* and *P. aureus* oviposited significantly earlier than those fed on *H. annuus* and *S. vulgare* (17.3, 17.7, 20.7 and 24.3 days, respectively) (Table 1).

A. hypogaea-fed adult females had the maximum fecundity (230.3 eggs/female), followed by those fed on *P. aureus* (218.7 eggs/female). The mean fecundity was 204.0 eggs/female and 168.7 eggs/female in those fed on *H. annuus* and *S. vulgare*, respectively (Table 1). It was maximum of 249.0 eggs in *A. hypogaea*-fed females to minimum of 159.0 eggs in *S. vulgare*-fed females. In the latter case, the maximum number of eggs laid by a female was only 179. The embryonic development duration was not significantly different in the eggs oviposited by females fed on four host diets (Table 1). Maximum hatchability was recorded in the eggs laid by females fed on *A. hypogaea* (87.6%), while the least was in the eggs laid by those fed on *S. vulgare* (74.8%) and these were significantly different.

Results from the analysis of some basic physical and chemical characters of the four host plant species are provided in Table 2. Leaves of *H. annuus* and *S. vulgare* were thicker and had more trichomes than *A. hypogaea* and *P. aureus*. Nutritionally, *A. hypogaea* and *P aureus* contained more proteins and nitrogen, in addition to the former having high lipid content and the latter carbohydrates. *A. hypogaea* had the least of the total carbohydrates and high levels of phenols amongst the four hosts, while *P. aureus* was a poor source of lipids, only better than *S. vulgare*.

Food has a direct effect on nymphal development, mortality, fecundity and longevity, which in turn controls the grasshopper populations. Food quality (Ananthakrishnan *et al.*, 1985) and availability (Haniffa and Periasamy, 1981) can influence developmental rate. In hemimetabolous insects, the number of instars vary from two to eleven (Wigglesworth, 1965), but many species commonly have four to six instars.

In *D. pinguis*, both sexes had same number of instars during nymphal development, while in some species like *Acrida exaltata* (Suresh and Muralirangan, 1996) female nymphs have an additional instar than males.

Fecundity is influenced by both genetic and environmental factors (Joern and Gaines, 1990). Insects feeding on high quality plants often have increased survivorship and fecundity (Mattson and Haeck, 1987) and the insects had low reproductive potential when fed on less nutritive diets (Chapman *et al.*, 1979 and Slansky, 1980). Fecundity was significantly higher in *D. pinguis* fed on *A. hypogaea* or *P. aureus*, which were nutritionally richer than *H. annuus* or *S. vulgare*. Factors like preovipositional period, incubation period and hatchability also had similar patterns in *D. pinguis*.

In *D. pinguis* the mortality was high during the first instar stage, and steadily declined upto the third instar stage, beyond which no mortality was observed, as in several other species (Waloff, 1972). In *D. pinguis*, the adult sex ratio was around 1 : 1, as in many other grasshopper species (Pickford, 1962; Pfadt and Smith, 1972).

In spite of lower carbohydrate (17.1 mg/g) and higher phenol (24.8 mg/g) contents, the leaves of *A. hypogaea* provided higher survival probability for *D. pinguis* than the leaves of *H. annuus* and *S. vulgare*. This may be due to the higher protein (92.4 mg/g) and lipid (230.2 mg/g) contents, lesser trichomes and thinner leaves which trades-off for the deficiency and deterrence in *A. hypogaea* leaves. Similarly, *P. aureus* has many favourable features like higher carbohydrate, protein, lipid and lower phenol contents which enhance the survivability of *D. pinguis*.

D. pinguis has a shorter nymphal, preoviposition and incubation periods, higher fecundity, adult longevity and hatchability, and lower nymphal mortality when fed on *A. hypogaea* or *P. aureus*, compared to those fed on *H. annuus* or *S. vulgare*.

ACKNOWLEDGEMENT

The first author thanks Dr. Niranjan V. Joshi for helping with analysis of the data and Dr. V. R. Bhagwat and Dr. A. John Peter for their helpful comments on the manuscript. This work was funded by University Grants Commission, Government of India. {Project No. F3/188/90(SR-II)}.

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(Received in April 1999; revised and accepted in August 1999)

ENTOMON 24(4): 359–362 (1999)
Article No. ent.244409

Postembryonic Observations on Two Spotted White Spider Mite *Tetranychus hypogaea* Gupta—a Pest of Groundnut

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ABSTRACT: The biology of the white spider mite *Tetranychus hypogaea* Gupta, a pest of groundnut, was studied under laboratory conditions. The egg period was 3.04 days, Ist to IIIrd instar of male and female were 1.39 ± 0.56 and 1.27 ± 0.47; 0.4 ± 0.46 and 1.00 ± 0.61 and 1.61 ± 0.68 and 1.15 ± 0.44 days, respectively. The total life cycle was 10.15 and 11.15 days respectively, for male and female. The female laid an average of 43 eggs in its life period and the reproduction was both by sexual and parthenogenetic methods. Male:female ratio was observed to be 0.03 : 1 and the males of this species feed on female of its own species and rarely on red spider mite (*Tetranychus cinnabarinus* Boised).

KEYWORDS: Biology, two spotted white spider mite, groundnut.

INTRODUCTION

The two spotted white spider mite, *Tetranychus hypogaea* Gupta was recorded as a pest of groundnut (Amin, 1988). These mites suck the sap, and the spots where the mite probed became light yellowish and finally became white. Due to feeding by heavy populations of mite on the leaflet, the leaf became stippled leading to yellowing and finally dries up. Apart from the informations, no observations were reported on this mite in groundnut. We report the Postembryonic observations of this mite on groundnut for the first time.

MATERIALS AND METHODS

Biology of the *T. hypogeae* was studied in detail in the laboratory at 30.37 ± 2.27 °C and 80.86 ± 12.03% RH. A filter paper (whatman No. 1) of the same size was lined inside the petridish of 4 cm diameter moistened and used as culture cells. A single leaflet of +3 or +4 leaves of the main branch of cv. Girnar 1, raised in earthern pot, was placed on the filter paper, keeping the upper surface upwards. A single ovipositing

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TABLE 1. Post embryonic observations on the two spotted white spider mite *T. hypogaea* on groundnut

Instar	Number of individual observed	Range (Days)	Mean ± SD (days)
Egg	25	2 to 4	3.04 ± 0.54
Male:			
Larva	14	12 h to 2.12	1.39 ± 0.56
Protonymph	14	12 h to 2.12	1.04 ± 0.46
Deutonymph	14	12 h to 3.00	1.61 ± 0.68
Adult	14	2.00 to 5.00	3.07 ± 0.92
Total Life Span	14		7.11 ± 2.62
Female:			
Larva	26	12 h to 2.12	1.27 ± 0.47
Protonymph	26	12 h to 3.00	1.00 ± 0.61
Deutonymph	26	12 h to 2.00	1.15 ± 0.44
Adult	26	3.00 to 8.00	4.69 ± 3.04
Pre-oviposition	26	12 h to 3.00	1.46 ± 0.69
Oviposition	26	1.00 to 5.00	2.46 ± 1.03
Post-oviposition	26	1.00 to 3.00	1.77 ± 0.76

female, from isogenic culture maintained in the laboratory, was released on each of the leaflet. Adequate water was added in the petridish so that the leaflet kept floating which will avoid movement of mite away from leaflet. Altogether twenty petriplates with ovipositing female were maintained. The eggs laid by each female was removed to similar petridish with single leaflet and numbered upto fifty. For further recording on the mortality and other observations, old leaflets were replaced with new ones after every 24 h. A reference culture was also maintained separately for morphometric measurements of different instars. The egg period was assessed from the time of egg laying to hatching of larva and like this, periods for different instars were recorded for both the sexes. The mating, moulting and feeding and such other observations were made. Using caliberated occular micrometer, the morphometrics were measured.

RESULTS AND DISCUSSION

The mite *T. hypogeae* feeds by probing its stylet into the leaf tissue and suck the sap. The spots where the mite probed became light yellowish and finally became white. Due to feeding by heavy populations of mite on the leaflet, the leaf became stippled leading to yellowing and finally dried up. Extensive webbing was also seen on the growing tip of groundnut shoot where thousands of mite congregate. The durations of various stages of male and female are shown in Table 1. The measurements of the various stages are given in Table 2.

TABLE 2. Measurement of different stages of the two spotted white spider mite *T. hypogaea* on groundnut

Instar	Mean (mm) of length and width	
	Male	Female
Egg	0.10	
Larva	0.15 × 0.09	0.25 × 0.20
Protonymph	0.18 × 0.12	0.30 × 0.25
Deutonymph	0.24 × 0.14	0.45 × 0.30
Adult	0.30 × 0.20	0.50 × 0.35

Egg

The eggs were laid singly on both the surfaces of a leaflet and covered partially with webbing. The eggs were dull white in colour, round in shape and measured 0.11 mm diameter. The egg period lasted for 3.04 ± 0.45 days.

Larva

The larva is the stage between emergence of neonate nymph from egg and the first moulting. There was not much variation on the duration for both male and female. The larva of both male and female possessed three pairs of legs. The male measured 0.15 mm×0.09 mm in length and width while female measured 0.25 mm×0.20 mm length and width. There was no difference in size and shape of the instar of male and female at this stage. Moulting had taken place in a peculiar way. When he larva started moulting, it stopped movement and fixed its stylet into the leaf tissue for 2–3 hours. When the new culticle fully formed, the old ones started loosening from the body and due to the pressure exerted by the larva, there was break of old exuviae on the middle of the dorsal side or near the propodosomal region. When the old cuticle breaks, the larva removes it stylet from plant tissue and moves away in search of site for feeding. The kind of moulting process was observed in all the instars of this mite.

Protonymph

The time taken to complete this stage was 1.04 day and 1 days respectively for male and female. The size of the female nymph increased by 20% while apparantly there was no difference in size of the male nymph. The male nymph measured 0.18 mm length and 0.12 mm width while the female nymph measured 0.30 mm length and 0.25 mm width.

Deutonymph

The female nymph attained considerable increased in size by 50% as compared to its preceding stage. The abdomen of female of this stage was almost round probably due to ovarian development. The males, however, increased in size by 30%. The male

exhibited clear difference from female in size and abdomen tapering towards the anal end. The duration was 1.15 days an 1.61 days for female and male respectively.

Adult

The adult male lived for 3.07 days while the female lived on an average for 4.69 days which included reproduction stages such as pre-oviposition, oviposition and post-oviposition periods. Male:female ratio was 0.03 : 1.0. the mating pattern was similar to that of *T. cinnabarinus* (Nandagopal and Gedia, 1995) wherein the male moved just below between the hind legs and lifted its spermatodactyl part so that it comes into contact with female spermathecae. A single male mated several females and mating lasted from 25 to 50 seconds with an average of 36 seconds. The female laid eggs continously for a maximum of 5 days with a mean of 3 days. Maximum number of eggs (85%) were laid during first three days. A mean of 43 ± 8.97 eggs ranging from 25 to 51 were recorded from a single female. Rarely the male pierces its stylet into the abdomen of the female and suck the content for quite some time (4–7 minutes) and eventually the female dies. Similarly the male of this species attack the female of the red spider two spotted mite (*T. cinnabarinus*) and suck the body content resulting eventually in death of the female. The total life cycle from egg to death of adult after oviposition in case of female was 11.57 days while the male took 10.57 days. The adults of both male and female measured 0.3 mm$\times$0.2 mm and 0.5 mm$\times$0.35 mm of length and width, respectively.

ACKNOWLEDGEMENT

The authors are grateful to Dr. P. S. Reddy, former Director, for facilities and encouragement and Dr. M. Mohanasundaram, the then Professor and Head, Department of Entomology, Tamil Nadu Agricultural University, Coimbatore for identifying the mite and critically going through the manuscript.

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(Received in April 1999; revised and accepted in July 1999)

ENTOMON **24(4):** 363–369 (1999)
Article No. ent.24410

Occurrence of Entomopathogenic Fungi *Paecilomyces farinosus* (Holmskiold) Brown and Smith and *Zoophthora radicans* (Brefeld) Batko in the Field Population of *Plutella xylostella* L. on Cabbage

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ABSTRACT: A survey was conducted to collect entomopathogenic fungi on *Plutella xylostella* on cabbage in and around Bangalore. Two entomopathogenic fungi, viz., *Paecilomyces farinosus* and *Zoophthora radicans*, were isolated. *Paecilomyces farinosus* caused 9.1–16.7% mortality with parasitism by *Cotesia plutellae* ranged between 23.7–54.5% in Devanahalli and Vorthur-White field areas. Whereas, *Z. radicans* caused 33.3–68.6% of mortality and with 2.9–33.3% parasitisation by *C. plutellae* at Hessaraghatta.

KEYWORDS: *Plutella xylostella, Paecilomyces farinosus, Zoophthora radicans, Cotesia plutellae, survey*, epizootics, cabbage.

INTRODUCTION

The diamondback moth (DBM) *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), is considered to be the most destructive insect pest of cruciferous plants throughout the world, and the annual cost for managing it is estimated to be U.S. $1 billion (Talekar, 1992). This insect is believed to be the most universally distributed of all Lepidoptera (Meyrick, 1928) as quoted by Talekar and Shelton (1993). Cultivation of cabbage is hampered due to the incidence of DBM on cabbage in Bangalore (Nagarkatti and Jayanth, 1982). Krishnakumar *et al.* (1986) estimated 52% loss in marketable yield due to DBM attack on cabbage in India. To control DBM, farmers use large quantities of insecticides, often spraying 'cocktails' of chemicals, which has resulted in development of resistance to practically almost all categories of chemical insecticides, viz., organophosphorus compounds, carbamates and pyrethroids (Sun

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et al., 1986; Miyata *et al.*, 1986) including biological insecticide like *Bacillus thuringiensis* (Tanaka, 1990; Tabashnik *et al.*, 1990). As a result, it is increasingly difficult to control DBM under field conditions. As a part of the Ph.D. programme, a field survey was conducted in order to collect entomopathogenic fungi on DBM in cabbage in and around Bangalore, to make best utilization of the fungus for effective control of DBM on cabbage.

MATERIALS AND METHODS

Cabbage fields from Devenahalli, Chickkaballapura, Doddaballapura, Hessaraghatta, Vorthur-whitefield and Hoskote were surveyed for fungal affected larvae of *P. xylostella*. Out of the five areas surveyed the incidence of fungal diseases was noticed only in three places, viz., Devanahalli, Vorthur-whitefield and Hessaraghatta. An area measuring 10 × 20 sq. m. was earmarked in each field in order to study the intensity of the disease against *P. xylostella*. Each and every plant in the area was thoroughly checked for the diseased larvae. The observations on per cent mortality due to disease and parasitoid were recorded on different dates as given in Table 1. The diseased larvae were collected in screw cap vials and brought to the laboratory for further investigations.

The diseased larvae were surface sterilized with 0.1% mercuric chloride for few seconds and then thoroughly washed with sterile distilled water. The excess water removed by keeping the diseased larvae on Whatman filter paper. The diseased larvae were then cut into small pieces with the help of sterile razor blade and the bits aseptically transferred on to the Sabouraud maltose agar, enriched with 1% yeast (SMAY) slants with the help of sterile inoculation needle. The slants were kept at 27 ± 2 °C. Diseased larvae were also kept on moist filter paper in Petri dishes for mycelial growth and sporulation.

The spores from the fungus which grew on SMAY slants were washed with distilled water + 0.01% Triton x-100 and inoculated to the laboratory reared larvae of *P. xylostella* (I–II instars) through surface contamination technique with the help of camel hair brush.

The diseased larvae collected form cabbage field at Hessaraghatta did not show any mycelial growth on SMAY slants, but the fungal spores from the diseased larvae kept on moist filter paper in Petri dishes were washed similarly and inoculated to the laboratory reared larvae of *P. xylostella*.

The inoculated larvae, with two different entomopathogenic fungi, were kept separately on cabbage leaves in plastic containers of size 13.5 × 11.0 cm. provided with moisture and meshed lids.

Pathogenicity tests were conducted according to Kochs postulates. The symptoms and the feeding behaviour of the diseased larvae, symptoms on larval death, mycelial growth and sporulation were recorded. The fungal culture from agar slant and on dead larvae were examined under microscope. The morphological characters of the fungi and size of phyalides and conidia were recorded with the help of stage and ocular micrometer under phase contrast microscope.

TABLE 1. Occurrence of entomopathogenic fungi in the field population of *Plutella xylostella* on cabbage

Date of collection	No. of larvae collected (I–IV instars)	% parasitism by *Cotesia plutellae*	% mortality by fungus
a) *Paecilomyces farinosus*			
at Devanahalli			
September 15, 94	80	42.5	12.5
September 18, 94	60	43.3	16.7
September 24, 94	55	54.5	14.5
at Vorthur-Whitefield			
September 16, 94	102	45.1	11.8
September 19, 94	88	36.4	9.1
September 30, 94	118	23.7	10.2
b) *Zoophthora radicans*			
at Hessaraghatta			
September 21, 94	70	8.5	51.4
September 22, 94	50	8.0	44.0
September 23, 94	57	17.5	49.1
September 25, 94	185	23.7	40.5
September 27, 94	76	19.7	60.5
September 28, 94	39	20.5	64.1
September 29, 94	35	2.9	68.6
October 1, 94	20	10.0	40.0
October 4, 94	12	33.3	33.3

RESULTS AND DISCUSSIONS

Both the fungal pathogens were identified primarily based on morphological character as per Humber (1984). The final identity of the fungi was confirmed by International Mycological Institute (IMI), London. They were identified as *Paecilomyces farinosus* (Holmskiold) Brown and Smith - the fungus collected from Devanahalli and Vorthur-Whitefield and *Zoophthora radicans* (=*Entomophthora sphaerosperma*) (Brefeld) Batko. - the fungus collected from Hessaraghatta (IMI accession Nos 356239 and 359439, respectively).

The occurrence of *Z. radicans* was more severe than *P. farinosus* (Table 1). The incidence of *P. farinosus* on *P. xylostella* was 9.1–16.7% and that of *Z. radicans* was 33.3 to 68.6%, and it was directly related to the temperature and humidity prevailed in the areas (max. 26–30 °C and min. 17–20 °C; RH during day: 61–91% and night: 51–83%). Rain also played very important role in case of epizootic of *Z. radicans*. Epizootic in *Z. radicans* was observed immediately after rain (26.4–

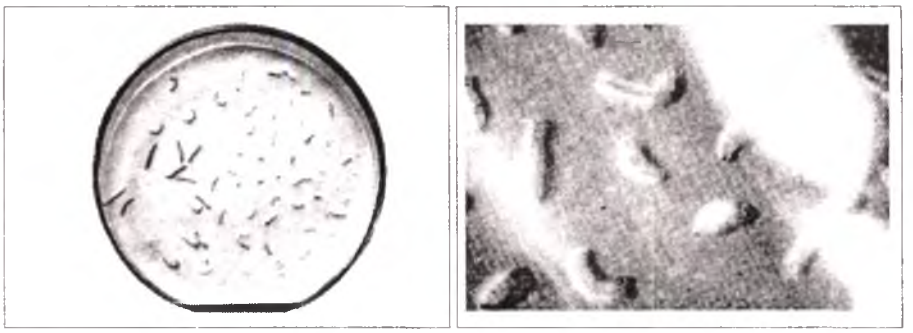

FIGURE 1. Effect of *P. farinosus* on *P. xylostella*. a. *P. xylostella* larvae affected by *P. farinosus* (H: Healthy, D: Diseased). b. Close up of fungal affected *P. xylostella* larvae showing white mycelial growth.

81.6 mm). Similar incidence of *Z. radicans* on *P. xylostella* immediately after rain was noticed by Riethmacher *et al.* (1990) supports the findings.

High population of indigenous parasitoid during the epizootic period (Table 1) indicates that both the fungi did not affect the parasitism by *Cotesia plutellae* on *P. xylostella* under field condition, though the percentage parasitism was comparatively low in case of *Z. radicans*. Furlong and Pell (1996) observed in the laboratory experiment that *C. plutellae* was not susceptible to the fungus *Z. radicans*. Field observations made by Velasco (1983) on the cabbage pest *P. xylostella* at Baguio city in the Philippines, showed that the larval parasitoid, *C. plutellae* and the fungus *Z. radicans* were the main biotic mortality factors. Thus, the natural percentage parasitism recorded at Devanahalli and Vorthur-Whitefield area ranged between 23.7 and 54.5% (Table 1) and that recorded at Hessaraghatta was between 2.9–33.3% (Table 1).

In comparing the signs and symptoms, the fungal infected larvae of *P. xylostella* resembled to that of any other fungal infected lepidopterous larvae as reviewed by Madelin (1966) and Ferron (1978). Infected larvae fed normally in the initial phase of the disease but the appetite diminished 48 hr after inoculation. Between 48 and 55 hr after inoculation, the larvae became restless, unstable, lost balance and ceased feeding. Diseased larvae were sluggish and showed slight tactile movement of the body when touched with a needle at the last body segment. At death, which occurred normally between 48 and 72 hr depending upon the stage of the insect, the body became tough and mummified. Mycelial growth occurred from 6–12 hr after death and sporulation noticed at 24 hr after death or 92 hr after inoculation (Fig. 1a.b).

The fungus, *P. farinosus*, grew well on Sabouraud maltose agar + Yeast (SMAY). The colonies had a tough mat basal felt and a loose hairy hyphal growth with spores brown in colour. The phialides were roughly flask shaped; 5–12 μ long and 1.5–2.0 μ diameter. Conidia are hyaline, broadly eliptical, 2.5–3.8 μ × 1.2–2.0 μ and were in short chains. The description of the fungus is similar to the descriptions given by Brown and Smith (1957) and Nene (1973).

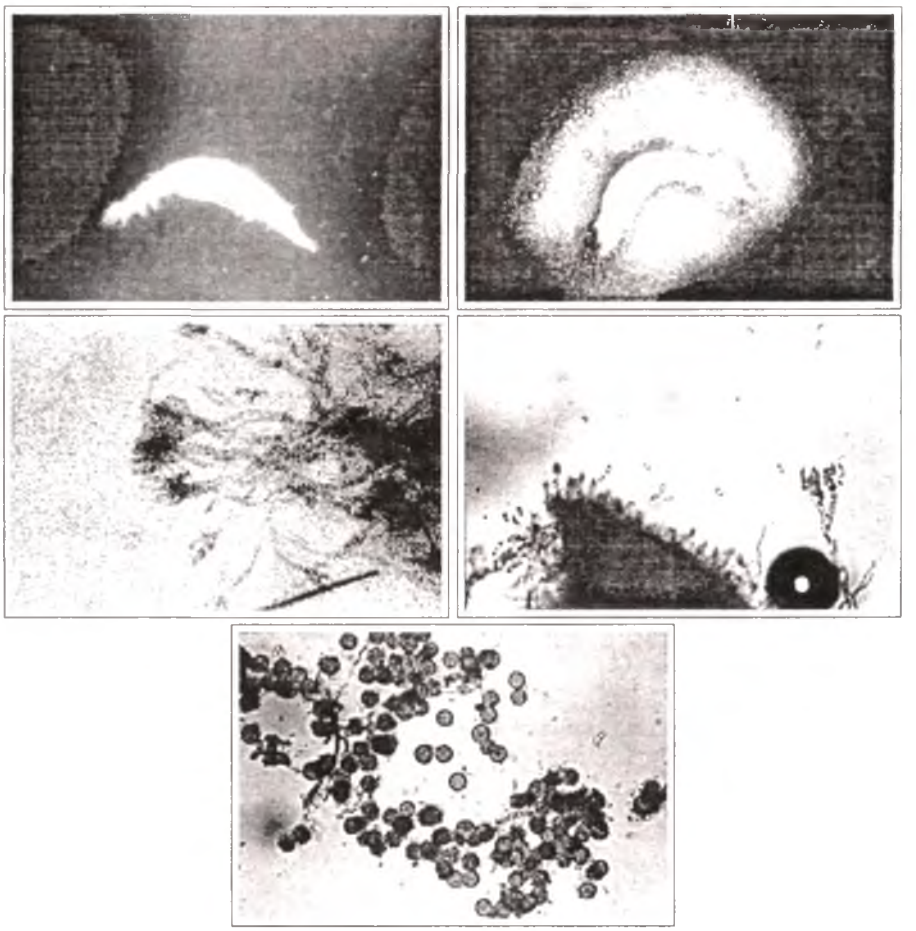

FIGURE 2. Effect of *Z. radicans* on *P. xylostella*. a. *Z. radicans* infected *P. xylostella* larva. Note the rhizoids of the fungus between the abdominal prolegs. b. *Z. radicans* infected larva showing the spores forming a zone around the body. c. The mycelium of *Z. radicans* showing conidiophore. d. The binucleated spores of *Z. radicans*. e. Resting spores of the fungus *Z. radicans* inside the diseased caterpillar of *P. xylostella*.

The fungus *Z. radicans*, which belonged to entomophthorales group, did not grow on SMAY. The larvae infected by *Z. radicans* became mummified and slightly hard with change in colour from green to yellowish. The fungus with the help of rhizoids fixed to the leaf substratum along with the larva (Fig. 2a). The fungus forms characteristic spore zone around the infected larvae (Fig. 2b). The spores which are usually binucleate are produced on the conidiophore (Fig. 2c,d). The fungus is also known to produce resting spores (Fig. 2e) inside the body of the diseased larvae to tide over adverse conditions.

The entomopathogenic fungus *P farinosus* was first recorded on cotton whitefly *Bemissia tabaci* Genn. in India from Pantnagar by Nene (1973) and subsequently on mango leaf webber *Orthaga exvinacea* H. by Rajan Asari *et al.* (1977), on cassava whitefly by Palaniswamy and Pillai (1984) and on *Eligma narcissus* by Mohanan and Varma (1988) from Kerala. The fungus *P. farinosus* was recorded for the first time on cabbage diamondback moth *P. xylostella* during the present study, and forms the first report in the world.

Whereas, the epizootic of *Z. radicans* on *P. xylostella* has been noticed in South Africa (Ullyett, 1947), Malaysia (Ooi, 1979), New Zealand (Kelsey, 1965) as cited by Muckenfuss *et al.* (1990) and in Philippines (Riethmacher *et al.*, 1990). Hence, the present record of *Z. radicans* on *P. xylostella* forms the first report from India.

The entomopathogenic fungi, *P. farinosus and Z. radicans* are considered to be natural biological control agents of *P. xylostella*, and the best utilization of these fungi in the management of DBM on cruciferous crops depends on the successful large scale multiplication of the fungi under laboratory conditions.

ACKNOWLEDGEMENTS

The first author is grateful to the Director, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, for granting study leave to undertake Ph.D. programme and the Head, Department of Botany, Bangalore University, Bangalore, for providing facility to carryout the study. The author is also grateful to Mrs. A. E. Eldrige, Identification Services, International Mycological Institute, London, for identifying the fungal pathogens.

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(Received in February 1999; revised and accepted in October 1999)

ENTOMON 24(4): 371–379 (1999)
Article No. ent.24411

Distribution of the Coconut mite *Aceria guerreronis* in Peninsular India and adjacent islands

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ABSTRACT: Investigation on the incidence, distribution and damage caused by the coconut mite *Aceria guerreronis* Keifer in Peninsular India and Sri Lanka has been carried out. The study has indicated establishment of the pest in the entire state of Kerala, Tamil Nadu and parts of Karnataka and spreading along the Western coast of Sri Lanka towards south. Very recently incidence of the mite has been recorded in three islands of Lakshadweep namely Minicoy, Kalpeni and Kavaratti. Analysis of the symptoms of injury caused by the mite has indicated drying and premature fall of nuts associated with nut cracking and deformation. The mite has attained devastating dimension throughout the area of its distribution. Tentative estimates have indicated 200 to 250 crores of rupees annual loss in the state of Kerala alone due to this mite. Meanwhile an effective control measure against the pest has not been attained so far. Therefore, an integrated approach to combat the epidemic is warranted as a future step to save our coconut plantations.

KEYWORDS: Coconut, *Aceria guerreronis*, invasion, Asia, injury, distribution.

INTRODUCTION

Coconut palm is a traditional crop plant of Indo-Malaysian region and constitutes an important cash crop of Peninsular India and neighbouring islands. The eriophyid mite *Aceria guerreronis* has been reported as a pest of coconut from the coconut belt of Americas and West Africa since last two decades (Hall and Espinosa, 1981). However, presence or pest status of this mite has not been felt in Indo-Malaysian region, eventhough this region forms the original place of this palm (Moore and Howard, 1996). Astonishingly, presence of the mite has been detected from Ernakulam district of Central Kerala during the end of 1990s (Sathiamma *et al.*, 1998). Following this, ravages of the mite have been detected at far and wide localities of Southern India (Haq, 1999), including Kerala, Tamil Nadu and Karnataka. Now the pest has extended its distribution to Sri Lanka and Lakshadweep islands creating pulse to a greater extent. Considering the practical significance and seriousness of the situation an analysis of the problem and future strategies to combat the situation is being made here.

MATERIALS AND METHODS

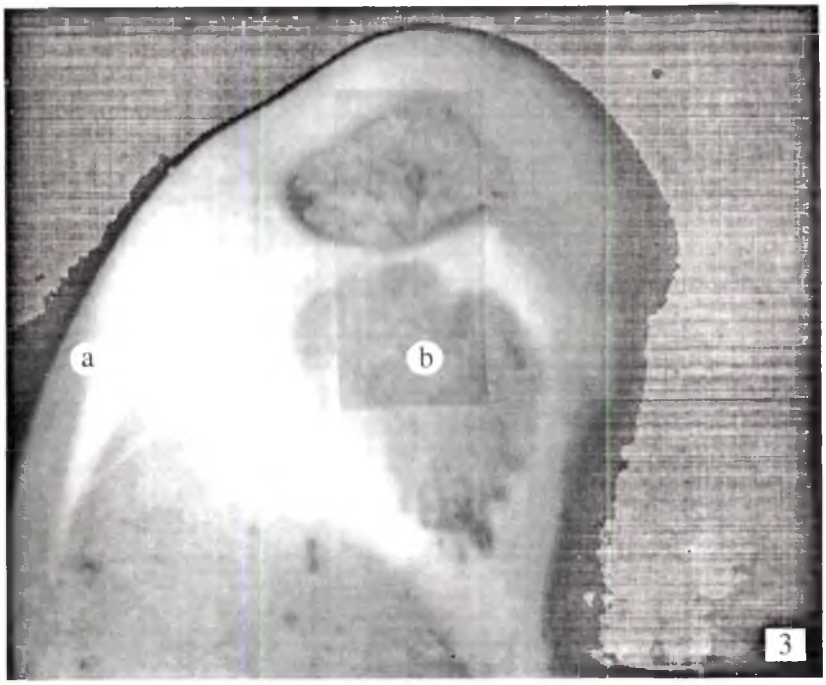
Samples of young coconuts aged 8 to 12 weeks showing symptoms of injury by *A. guerreronis* were collected from coconut plantations of Kerala, Karnataka, Tamil Nadu, Sri Lanka and Lakshadweep islands for making comparative analysis of the injury caused. The nuts were subjected to microscopic examination to screen the colonies of mite on them. The tepals of individual nuts were removed carefully and the number of mites/square centimetre area of the meristamatic region was counted. Symptoms of damage associated with infested nuts from various regions were analysed for comparative account. The sequential changes of infested nuts in the field were observed and recorded on selected palms from localities around Calicut University Campus. Loss in crop yield in Kerala was tentatively estimated through information gathered from respective agricultural offices and personal discussions with farmers and scientists.

RESULTS AND DISCUSSION

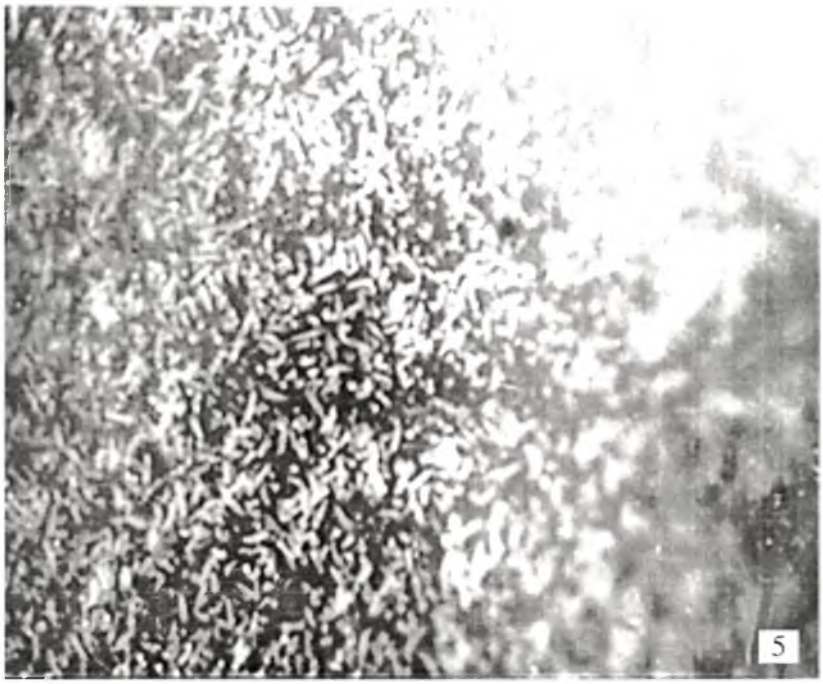
Incidence of *A. guerreronis* and associated symptoms on developing nuts have been evident in all the districts of Kerala now. However, the extent of devastation appears to be varied on close scrutiny. The distribution of the mite in various districts has been studied and accordingly they were categorised as High, Medium, Mild and Rare based on degree of infestation (Plate 1). After making sporadic appearance at Elanchi Vytila and Amballore area of central part of Kerala belonging to Ernakulam district, the mite migrated to Allappuzha and subsequently to Kottayam, Thrissur and Palakkad districts more or less simultaneously. Then it performed a southern journey to Thiruvananthapuram and Northern journey to Kasargod covering the districts Kollam, Pathanamthitta, Idukki, Malappuram, Kozhikode, Kannur and Kasargod. Apart from this, mite infestation has been established along the coastal belt of the Dakshin Kannada and Bangalore in Karnataka. Invasion by the mite to Tamil Nadu occurred at Coimbatore from where it, enchroached other districts, currently covering the entire state. (Dr. Mohanasundaram, Personal Communication). Presence of the mite in adjacent islands like Sri Lanks and Lakshadweep has been detected by the author. Northern, northcentral and northwestern provinces of Sri Lanka are found affected by the mite. Of these, few regions of Northwestern province are found badly affected. However, spread of the mite towards the Northcentral and western provinces is well documented through detection of flourishing colonies of the mite on palms in and around Colombo city. During recent sampling from Lakshadweep islands, presence of the mite has been recorded from Minicoy, Kalpeni and Kavaratti. This being the first report of *A. gurreronis* from the above islands (plate 1), it warrants the necessity of urgent steps to be initiated for controlling the mite, as coconut forms a major source of income for these island people.

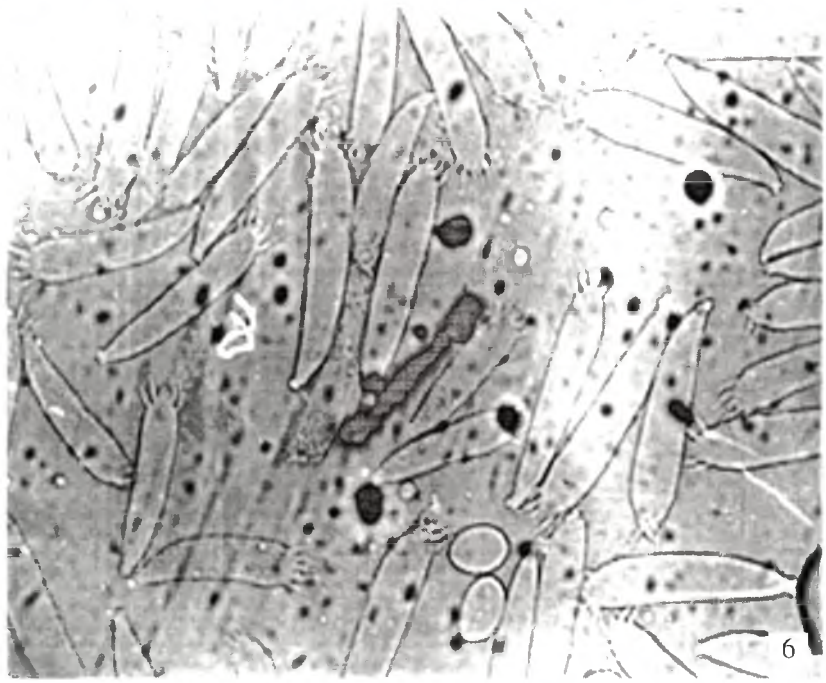
Screening of affected nuts has revealed various types of symptoms on them. Presence of mites has been detected on nuts of later stages of development. (Haq, 1999). However, symptoms of injury persist even after the abandonment of the nuts

FIGURES 1–2. 1: A coconut palm with bunches of nuts showing various degrees of infestation by *Aceria guerreronis*; 2: An infested tender coconut showing yellowish white and brownish triangular patches.



FIGURES 3–4. 3: Close-up view of the infested nut after removal of perianth. (a) fresh triangular patch (b) mite fed area of the meristematic tissue; 4: mature coconuts exhibiting varying degrees of damage by *A. guerreronis* along with an uninfested nut harvested from the same tree.





FIGURES 5–6. 5: Replenishment of colony of *A. guerreronis* under the perianth; 6: A slide mounted view of various life stages of *A. guerreronis*.

by the mite. Evidently the affected nuts carry permanent deformities. Initiation of mite infestation occurs on nut of 4 to 6 weeks of age. Some of the affected nuts exhibit arrest of growth at this stage. A large percentage of such nuts dried up slowly and retained on the bunch (Fig. 1). However, some of the infested nuts continue to develop even after mite invasion. At this stage, the nuts reveal the presence of triangular white patches extending from the lower edge of the perianth. Prolonged feeding of the mites result in extension of the patch further down which can easily be differentiated from the fresh feeding patch by its length and width (Fig. 2). Such patches turn dry exhibiting brownish colour in due course. The triangular white patch appears to be extension of feeding area of meristematic tissue with light yellow and brown markings on them which can be perceived on removal of the perianth (Fig. 3). Slowly the region of feeding patch dries up and develops small fissures and streaks. The husk turns very hard and thin. As the nut grows, these brown patches enlarge and almost cover the entire length of the affected nut. Most of the nuts develop irregular foldings on the husk showing highly deformed appearance (Fig 4). The affected nuts never attain normal size and shape.

Microscopic examination of the affected nuts at various stages of infestation has revealed the presence of mites on nuts of 4–24 weeks of age. Throughout this period the mites remain under the perianth and develop extensive colonies (Fig. 5) at the meristematic zone of the nuts. Such colonies comprise all life-stages of the mite from egg to adult (Fig. 6). The number of mites per square centimetre area of the meristematic zone during peak period of infestation reached more than 1200 under Kerala conditions.Infested nuts of 3 to 4 months of age from Sri Lanka and Lakshadweep showed an average of 600–800 and 200–300 mites per square centimetre of the meristematic zone respectively. Presence of mites out side the perianth occurs only at the advanced stage of infestation. This marks dispersal phase of the mite when large number of adults are found roaming on and around the perianth.

A. guerreronis, which appeared sporadically during early 1998 in few localities at the central part of Kerala has gained momentum within a couple of months and attained the status of a serious pest dramatically. Reports on the spread of the mite from central Kerala (Haq, 1999, 2000; Haq *et al.*, 2000) encroaching all other regions of the state threaten all sectors of people, owing to the failure of control measures in the state. While considering the current distribution pattern of the pest in India, it is apparent that the pest has succeeded in establishing itself along the entire range of its migratory path. Such rapid spread of the mite has been reported earlier (Doreste, 1968; Mariau, 1986). Therefore it is reasonable to attribute tremendous invasive and establishment powers to the mite, which in turn has raised it to the status of a cosmopolitan pest throughout the coconut belt of the world.

On the basis of the symptoms of injury observed in the field, it can be concluded that *A. guerreronis* inflicts a series of damage to developing nuts. Of these, early drying of nuts, though observed on infested palms frequently, more specific studies have to be conducted to confirm the role of this pest in causing such change in the nuts. All the other kinds of damages appear to be of coconut mite infestation. Of

DISTRIBUTION OF *ACERIA GUERRERONIS* IN KERALA AND NEIGHBOURING ISLANDS.

these, crack development followed by premature fall of the nuts appears to be the most severe manifestation, as this results in complete destruction of the affected nuts. However, extent of damage on nuts attaining maturity, still surpasses the level of minor injury. Almost all the infested nuts attaining maturity are smaller in size, with drastic reduction in their volume (Haq, 2000).

While considering the infestation of *A. guerreronis* on developing nuts of coconut palm, it is quite evident that the mite gains success due to its well protected and secluded microhabitat and unperceivable way of dispersal. Wind current has been

suggested as the carrier of this mite (Moore and Howard, 1996; Ramarethinam and Marimuthu, 1998). However, it is apparent that the mite depends on some other means for dispersal as well (Moore and Howard, 1996). It is quite reasonable to attribute such possibilities while considering the spread of the pest in Kerala, Sri Lanka and Lakshadweep islands. Human activities like transportation of materials, particularly tender nuts may facilitate establishment of the mite population in the new areas. More studies are needed to unravel the mechanism of dispersal of this pest to evaluate strategic regulatory measures.

Possibilities of indiscriminate usage of nitrogen fertilizers leading to loss in resistance of the palms cannot be ruled out. It is known that surplus quantity of nitrogen and deficiency of potassium may invite plant feeding forms and may lead to replenishment of mites. Potassium being a highly essential element imparts much resistance to the palms from insects and non-insect pests (Mandal, 1991). Non availability of this element in required quantity coupled with prolonged dry spell experienced during the last few years and elimination of natural predators due to heavy pesticide application might have shared their role collectively in contributing the current outbreak and havoc, created by the mite pest.

Sequence of arrangement of tepals on developing nut and their mode of attachment to the meristamatic zone have been found as deciding factors for mite invasion (Moore, 1986). Loose arrangement of tepals, particularly with overlapping ends on adjacent ones while favouring mite entry, tightness with either ends overlapped by the adjacent tepals would reliably check easy accessibility of the mite. This would advocate selection of tight fitting tepal variety of coconut for planned farming system in future. However, frequent development of splits and crevices so common on free lower border of teplas often provides sufficient space for mite invasion. Better cultivation practices and application of adequate fertilisers seem to minimise chances of mite invasion (Romney, 1980). All the above points would further indicate the urgent need for indepth search on varietal tolerance of palms to mite invasion.

A. guerreronis has become a problem in countries where it has been identified as a pest, but the degree of crop loss appears to be of high order in Peninsular India where coconut has attained the status of chief plantation crop. This has elicited considerable economic loss as it is now reached to the tune of about 200–250 crores of rupees per year in Kerala alone. The fact that this plantation crop constitutes one third of the income of the state further warrants the extreme necessity of an integrated approach to be streamlined for effective regulation of the mite pest in the light of our unsatisfactory control efforts. Expertise available in this regard need to be detected and utilised for better formulation of effective control strategies for safe-gaurding our 'Kalpavriksha' and in turn the people of Kerala.

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(Received in November 1999; revised and accepted in January 2000)

ENTOMON 24(4): 381–383 (1999)
Short Communication No. ent.24412

Nematode Provoked Prophenoloxidase System Inducing the Humoral Encapsulation in *Halys dentata* (Hemiptera-Pentatomidae)

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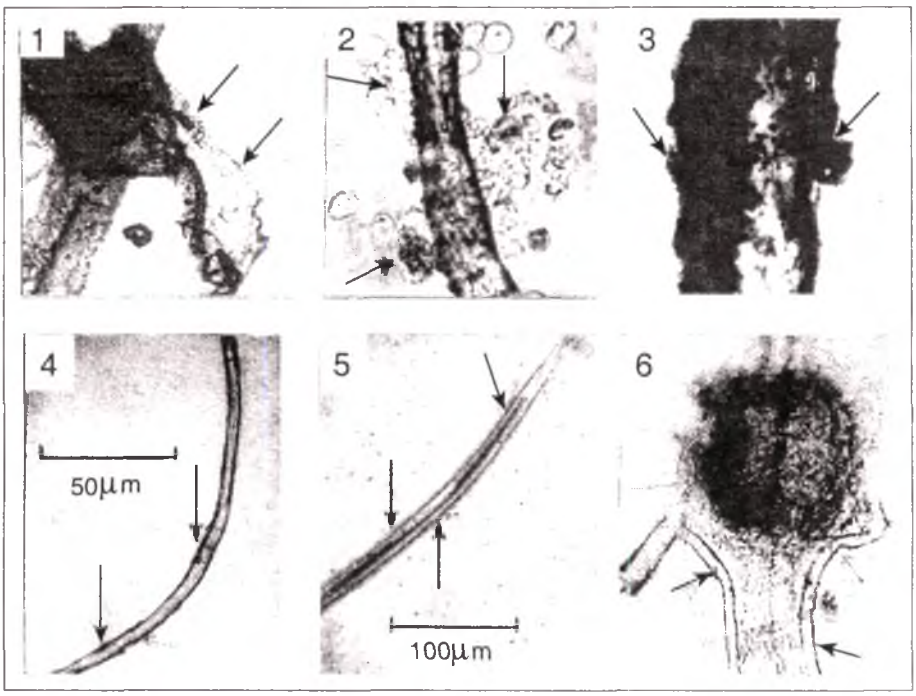
ABSTRACT: *Halys dentata*, a pentatomid plant bug showed cellular encapsulation of biotic and abiotic nonactive objects as a normal defense mechanism. When the dauer larvae of *Neoaplectana carpocapsae* were implanted in the haemocoel of adult insects, the prophenoloxidase activity was provoked due to active lysis of granular haemocytes. The activation of prophenoloxidase- phenoloxidase implicated in humoral encapsulation of parasite and other objects when implanted with the parasite. The activity was temperature dependent and also observed *in vitro*.

KEYWORDS: Prophenoloxidase, Humoral encapsulation, *Halys dantata*, Nematode.

INTRODUCTION

The humoral encapsulation is considered as the characteristics of dipteran insects in which some melanotic material precipitate from the haemolymph without visible participation of haemocytes (Bronskill, 1962; Vey and Gotz, 1975). The dipterans which show humoral encapsulation contain very low number of haemocytes (Gotz and Boman, 1985). In *Halys dentata* the haemocyte population varied from 9760 to 17800 mm3 (Bahadur & Pathak, 1971).

The nerve cord of *Galleria mellonela* (nonself i.e. allograft) showed the cellular encapsulation *in vitro* when treated with the haemolymph of *Halys dentata* (Figs 1 and 2). When the glass rod was implanted in the haemocoel of *Halys dentata*, it was completely encapsulated (Fig. 3) and melanised by the haemocytes within 24 hr. Thus it was concluded that in *Halys dentata* cellular encapsulation is the normal defense mechanism. However, when 10 to 20 dauer larvae of *Neoaplectana carpocapsae* were injected in the haemocoel of adult *Halys dentata* the humoral encapsulation was induced and the parasite was encapsulated by melanotic capsule without direct involvement of haemocytes (Figs 4, 5). Similar observations were also made *in vitro*. The process was temperature dependent, a complete capsule was formed within one hr. at 27°C while at 4°C it remained incomplete evenafter 48 hr. some times a nematode



FIGURES. 1–6. 1: Nerve cord of *Galleria mellonela* (non self i.e. allograft) showing the cellular encapsulation (*in vitro*) after 30 minutes when treated with haemolymph of *Halys dentata* (100 x). 2: Nerve cord of *Galleria mellonela* (non self i.e. allograft) showing cellular encapsulation (*in vitro*) after 2 hrs. When treated with the haemolymph of *Halys dentata* (200 x). 3: Cellular encapsulation of glass rod (*in vitro*) after 24 hrs. when implanted in the haemocoel of *Halys dentata* (100 x). 4: Humoral encapsulation of nematode *in vivo* in *Halys dentata* after one hr (100 x). 5: Humoral encapsulation of nematode *in vitro* in the haemolymph of *Halys dentata* after one hr. at 27 ° C (200 x). 6: Deposition of melanin particles on the surface of nerve cord of *Galleria mellonela* when implanted in the haemocoel of *Halys dentata* with nematodes (100 x).

was found to escape from the capsule. The melanotic crust used to adhere with other objects such as the nerve cord (Fig. 6) when implanted with the nematode in the haemocoel of the host.

The capsule was insoluble in pyridine, concentrated hydrochloric acid and sulphuric acid. It shows no colouration with PAS and blackened by ammonical silver nitrate suggesting the presence of melanin (Vey and Gotz, 1975).

The formation of capsule is dependent on phenloxidase activity as the process can be blocked by PTU and glutathion. The vigorous movement of nematode

probably initiates the fast lysis of granular cells. The lysed granular cells release prophenoloxidase, which is converted into phenoloxidase (Leonard *et al.*, 1985).

In *Halys dentata* the random but frequent contact between nematode and granular cells, thus initiates the release of prophenoloxidase cascade from granular cells which is activated to precipitate as melanin in haemolymph (Soderhall and Smith, 1986). It forms melanotic capsule or humoral encapsulation of nematode due to prophenoxidase present in the plasma of the haemolymph. It might be activated upon contact with *Neoaplectana carpocapsae* as suggested by Vey (1993). The inert implants perhaps fail to trigger of this mechanism.

The work is the part of the project No. F.17/85 RFII of University Grants Commission, New Delhi.

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(Received in April 1999; revised and accepted in August 1999)

ENTOMON 24(4): 385–387 (1999)
Short Communication No. ent.24413

Trachys sp. (Buprestidae: Coleoptera) as a Pest of Ladies Finger *Abelmoschus esculentus* in Kerala

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ABSTRACT: The buprestid beetle *Trachys* sp was found as a leaf miner of the vegetable crop, *Abelmoschus esculentus*. This is the first record of the pest from Kerala.

KEYWORDS: Buprestid beetle, *Trachys*, Leaf miner, ladies finger.

Ladies finger *Abelmoschus esculentus* an important vegetable crop of Kerala is infested by a variety of insect pests causing damage to leaves, stem, flowers and fruits. The leaf feeders of the plant commonly seen in Kerala are leaf roller, *Sylepta derogata*. semilooper caterpillars *Anomis flava* and *Acontia groelsi* and leaf weevil *Myllocerus sp.* (Nair, 1989). During the post monsoon season ie. October to December 1997, a buprestid beetle *Trachys* sp. could be observed in Quilon and Trivandrum districts of Kerala as a serious pest on ladies finger mining the leaves. The adults and grubs were seen feeding on the leaves.

The adult beetles are minute, flattened oval with dark blue metallic colouration. Elytra striated shiny and with golden wavy markings towards posteriorily. The size of the adult varies from 3.0 × 1.8 mm to 2.8 × 1.7 mm. Antennae are very short and the thorax is slightly elevated. The adult beetles are active and they damage the leaves by notching the margins (Fig. 1). Eggs are laid singly on the upper leaf surface near the margin. Many eggs are laid on a single leaf. Eggs hatch in 3–5 days. Newly hatched grubs are white in colour and flat. They mine the leaves and feed on the soft mesophyll, leaving only the white papery epidermal layers (Fig. 2).

Grubs remain within the mines that appear as irregular blotches on the lamina. Later the blotches shrivel up ultimately leaving an irregular hole on the leaf lamina. Several blotches can be seen in a single leaf each harbouring a grub within.

Grubs become full grown within a week. The full grown grub is 7–8 mm long, dorso-ventrally flattened with a broad head and the body tapering posteriorily. It is yellowish in colour with a dark spot on each segment dorsally. The grubs pupate within the leaf mine. The pupa is brown in colour 3–4 mm long oval in shape with the anterior end broader. The pupation lasts for 3–4 days. More than sixty per cent of leaves were seen infested during the peak period of infestation.

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FIGURE 1. Notching the leaf margins by beetles.

FIGURE 2. Mines on the leaf by grabs.

Other buprestid beetles reported as leaf miners of crops in India include *Trachys pacifica* on jute, *T. ipomoea* on sweet potato and *Trachys* sp on barleria (Nair, 1986; Nayar *et al.*, 1992). *T. virescense* is reported as a serious pest of ladies finger in Madhya Pradesh and Gujarat (Nair, 1986). Obeb (1985) has studied the biology of *T. herilla* as a leaf miner of Okra from Gujarat. So far there is no report of *Trachys* sp. from any host plants from Kerala. This is the first report of the beetle *Trachys* sp. as a leaf miner of ladies finger from Kerala.

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(Received in March 1999; revised and accepted in June 1999)

ENTOMON 24(4): 389–392 (1999)
Short Communication No. ent.24414

In Vitro Production of Conidia of Entomopathogenic Fungus *Paecilomyces farinosus* (Holmskiold) Brown and Smith

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ABSTRACT: A simple and cost effective technique for mass production of conidia of *Paecilomyces farinosus* under *in vitro* has been developed. Among the various cereals, brans, pulses, vegetables, roots, seeds and synthetic media tested, sorghum was found to be the best ideal and cheap media for the large scale production of conidia of *P farinosus*. 100 g of sorghum grain yielded on an average 10.41×10^{12} spores. Sabouraud maltose agar + yeast (SMAY) though yielded on an average 10.83×10^{12} spores/100 ml of the medium, cost and preparation wise sorghum was considered to be better than SMAY.

KEYWORDS: *Paecilomyces farinosus*, conidia, *in vitro* production, cereals, pulses, brans, vegetables.

INTRODUCTION

The entomopathogenic fungus, *Paecilomyces farinosus* (Holmskiold) Brown and Smith (Deuteromycetes: Moniliales) is a potential biocontrol agent of several lepidopterous insect pests (Prenerova, 1994). The effect of the fungus against cabbage diamondback moth, *Plutella xylostella* has been well established (Gopalakrishnan, 1998). Mass production of fungal pathogen is a prerequisite to take up any large scale field trial against the insect pest on cabbage. Hence, as a part of the Ph. D. programme, an experiment was carried out to mass produce the conidia of *P. farinosus* on various synthetic and natural media.

The various nutrient media, viz. cereals, pulses, brans, vegetables, root and seed listed in Table 1 were tested for the production of conidia of *P. farinosus*. Known quantity of each medium was dissolved in 250 ml sterile distilled water and allowed it to boil. After boiling the medium was poured into culture tubes of size 15.0 × 14 cm (8.0 ml/tube) and plugged with sterile cotton. The medium in the culture tube was then

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TABLE 1. Influence of different media in the production of conidia of *P. farinosus* under *in vitro*.

	Medium	Mean conidial production* ($X \times 10^{12}$ spores/100 g or ml)
1.	Finger millet	8.54
2.	Maize	6.66
3.	Paddy	9.16
4.	Pearl millet	8.54
5.	Sorghum	10.41
6.	Wheat	8.33
7.	Wheat bran	7.29
8.	Rice bran	8.12
9.	Bengal gram	7.29
10.	Horse gram	7.29
11.	Jack seeds	8.33
12.	Carrot	9.37
13.	Tapioca	6.25
14.	Corn meal agar	4.58
15.	Potato dextrose agar	9.37
16.	Sabouraud dextrose agar	9.37
17.	Sabouraud maltose agar	9.37
18.	Sabouraud maltose agar + Yeast	10.83
19.	Czepek Dox agar	3.33
20.	Potato carrot agar	9.37
	C.D.($P = 0.05$)	1.07

*Mean of three replications
Means were compared statistically by DMRT

autoclaved under 15 psi pressure at 121 °C for 30 min. Slants were made by arranging the tubes on a raised flat-form.

Ten tubes per medium were inoculated with fungal spores obtained from the culture originally isolated from diseased caterpillars of *P. xylostella* (Gopalakrishnan, 1998) and maintained on Sabouraud maltose agar + yeast (SMAY) slants. Inoculation was done using sterile inoculation loop under aseptic condition inside the laminar flow. The inoculated slants were incubated at 25 °C inside the BOD incubator.

Daily observations on mycelial growth and sporulation were recorded. Three slants, randomly selected from ten tubes, served as replications for each of the medium. The spores were washed from the slants on the tenth day of inoculation, with sterile distilled water containing 0.01% Triton x-100 using a glass rod and the suspension filtered through double layered muslin cloth. Counting of spores were made after serial dilutions of the suspension using double ruled Neubauer haemocytometer under phase-contrast microscope. The quantity of spores per slant determined after back calculation of the dilutions.

Three replications of 100 g each of overnight soaked cereals and pulses were taken

in separate 500 ml conical flasks. To each of the flasks 30 ml sterile distilled water was added and the flasks plugged with sterile cotton. The media in the flasks autoclaved under 15 psi pressure at 121 °C for 30 min. After cooling, the media was inoculated with the fungal spores obtained from SMAY slants using sterile inoculation loop (1 loop of spores/flask) under aseptic condition. The flasks after inoculation were kept in BOD incubator at temperature 25 °C.

Ten days after inoculation 10 g of the media was taken in a test tube and the spores washed with distilled water containing 0.01% Triton x-100 by thorough shaking. The suspension filtered through double layered muslin cloth. The spore suspension was subjected to counting and quantification as described above.

Wheat and rice brans were used to culture the fungus for spore production. Three replications of 50 g each of wheat and rice bran was mixed with 100 ml sterile dist. water and transferred to 500 ml conical flask. The flasks were plugged with sterile cotton. Sterilization and inoculation of the media, and quantifying of the spore production was done as described earlier.

The vegetables, viz, potato and carrot, and tapioca root and jack seed were also tested for the mass production of fungal spores. The vegetables, root and seed were peeled, cut into small pieces and washed thoroughly with distilled water. Three replications of 100 g of each media was taken in separate 500 ml conical flasks. 30 ml of sterile distilled water was added to the flask and the flask was plugged with sterile cotton. Sterilization and inoculation of the media, and quantifying of the spore production was done as described earlier.

The results presented in Table 1 showed that, among the cereals tested the production of fungal spores was significantly high on sorghum recording 10.41×10^{12} spores/100 g, followed by paddy which recorded 9.10×10^{12} spores/100 g. Finger millet, pearl millet and wheat recorded 8.54, 8.45 and 8.33×10^{12} spores/100 g and they were on par with paddy. The lowest production of 6.66×10^{12} spores/100 g was recorded on maize. Though wheat bran recorded low spore production of 7.29×10^{12} spores/100 g but it was statistically on par with the high spore production of 8.12×10^{12} spores/100 g on rice bran.

Among the pulses, seeds and roots tested carrot showed highest spore production of 9.37×10^{12} spores/100 g, followed by jack seeds which recorded 8.33×10^{12} spores/100 g. The production in both Bengal gram and horse gram was 7.29×10^{12} spores/100 g. Tapioca, however, recorded the lowest production of fungal spores (6.25×10^{12} spores/100 g).

Among the various nutrient media tested the highest production was observed on SMAY medium (10.83×10^{12} spores/100 ml). The other nutrient media Potato dextrose agar, Sabouraud dextrose agar, Sabouraud maltose agar and Potato carrot agar though recorded low production of spores of 9.37×10^{12} spores/100 ml of the medium, they were statistically on par with SMAY. Carrot maltose agar and Czapek Dox agar, however, recorded low production of conidia of 4.58 and 3.33×10^{12} spores/100 ml, respectively (Table 1).

Though the synthetic media, SMAY recorded high yield of conidia, but cost and

preparation wise sorghum works out to be cheaper and easier than SMAY (Cost of production of 1.04×10^{15} spores/1 kg sorghum = Rs. 5/- and Cost of production of 1.08×1015 spores/1 lit. of SMAY = Rs. 157.40, excluding over head charges). SMA was enriched by the addition of 1% yeast extract as yeast is considered to be a favourable nitrogen source for the production of large number of spores in the media (Latge *et al.*, 1978; Im *et al.*, 1988).

Paddy was also found to be suitable for the mass production of fungal spores and it was found best next only to sorghum (Table 1). The fungal spore of *Nomuraea rileyi* was successfully produced on polished rice (Silva and Loch, 1987).

The fungal spores were also been produced on wheat and rice bran. The production of spores on wheat was 8.12×10^{12}/100 g as compared to 7.29×10^{12}/100 g on rice bran. Ying (1986) cultured the fungus on medium containing both wheat and rice bran and by enriching with dry yeast and sugar. According to him the culture obtained on 1250 g of bran was diluted with 30 litter of water and applied to the pine plantations in China, which gave effective control over pine caterpillars.

Thus, from the above results it is concluded that among the cereals tested sorghum is very ideal and cheap media for the large scale multiplication of conidia of *P. farinosus* and among the synthetic media SMAY is the best media for laboratory culturing and maintenance of *P. farinosus*.

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(Received in February 1998; revised and accepted in October 1999)

ENTOMON 24(4): 393–395 (1999)
Short Communication No. ent.24415

Record of a Lac Insect, *Kerria* sp. (Homoptera : Kerridae) in Kerala

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ABSTRACT: The occurrence of a lac insect, *Kerria* sp. (Homoptera : Kerridae) on *Amherstia nobilis* Wall. (Leguminosae) is reported. Kerria sp. is a new record from Kerala and *A. nobilis* is recorded for the first time as host to a lac insect species.

KEYWORDS: Lac, Kerria sp., *Amherstia nobilis* Wall.

Lac is one of India's most important Non Wood Forest Products and about 85 per cent of the world's total output is grown in the country. Lac, a natural resin is the hardened secretion of the lac insects. This is further processed to make seed lac and shellac which are used in various products like paints, varnishes etc. The well known Indian lac insect *Kerria lacca* (Kerr.)(=*Laccifer lacca*) is used for commercial production of lac in India and other countries like Thailand, Philippines, Sri lanka etc. Several strains or varieties of *K. lacca* are known to exist with slight differences in their morphological and biological features. The host tress of *K. lacca* include *Shorea roxburghii* G. Don, *Accacia nilotica ssp. indica* (Benth.) Brenan(=*A. arabica* auct. non Linn.), *A. catechu* (Linn. f.) Willd., *Butea monosperma* (Lam.) Taub., *Ficus* spp., *Cajanus cajan* (Linn.) Millsp., (=*C. indicus* Spreng.), *Zizyphus mauritiana* Lam. (=*Z. jujuba*, (L.) Gaertn.), *Z. xylopyrus* (Retz.) Willd. etc. (Beeson, 1941). In India, Madhya Pradesh, Orissa Bengal and Bihar are the important lac production states.

Not much information is available on lac insects of Kerala. In a recent casual observation, a species of lac insect, *Kerria* sp. was recorded on *Amherstia nobilis* Wall. in Trichur, Kerala. *A. nobilis* known for its beautiful flower is a moderately sized evergreen tree species. It is indigenous to Myanmar and is usually grown in gardens in India and other countries (Anonymous, 1983). Among the three tress grown in the Trichur Zoo campus, one tree was found bearing lac insects in the month of January. Lac encrustations of 2–3 cm in thickness and 10–15 cm in length were found on twigs of many branches of the tree. The cylindrical and elongate encrustations were fresh and well developed with life stages of the insect (Fig. 1) developing inside. The infestation begins with the young larvae establishing on tender branches. They feed on the sap

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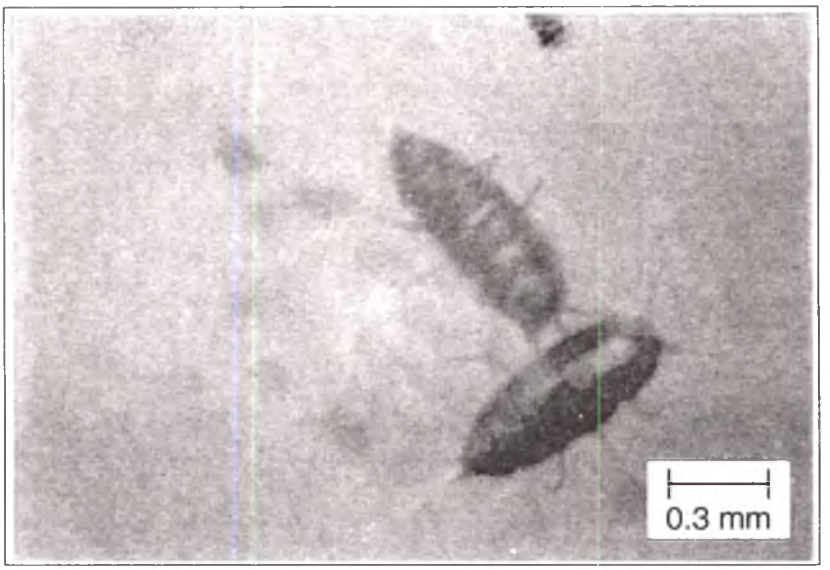

FIGURE 1. Microphotograph showing larvae of *Kerria* sp.

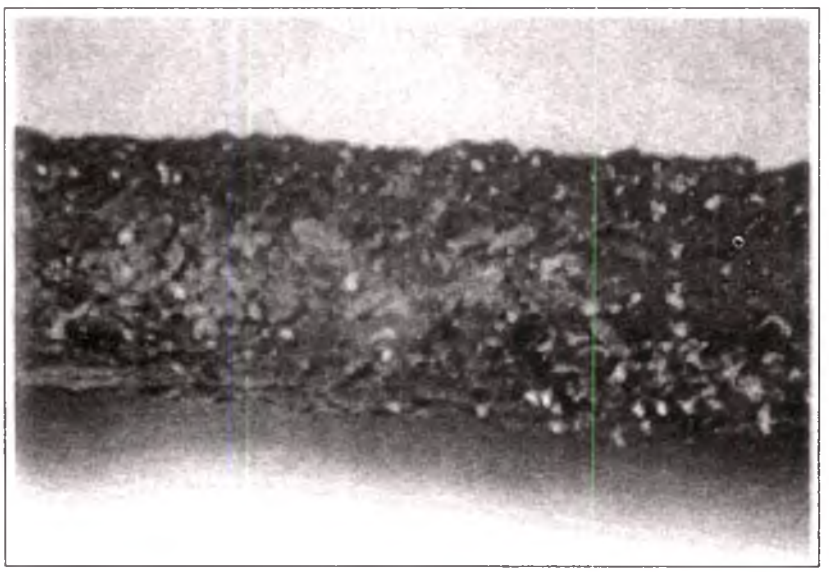

FIGURE 2. A developing lac encrustation on a twig showing active larvae.

of the tree and develop and in due course secrete a resin which covers their body as protective layer which gradually develop into lac encrustations over the infested branches (Fig. 2).

Inspite of the presence of many suitable hosts, there is no previous record of lac insect from Kerala. Hence, the present finding suggests the need for a detailed investigation on this commercially important group of insect in the state.

The production of lac depends on the intricate relationship between the species and the host tree. Hence, detailed studies are required to establish the importance of the lac insect species reported here for its commercial exploitation.

ACKNOWLEDGEMENTS

We are grateful to Dr. K. K. Kumar, India Lac Research Institute, Ranchi and Dr. N. Sasidharan, Kerala Forest Research Institute for identifying the insect and the host tree respectively. We thank Mr. K. V. Raveendran, former Superintendent, Trichur Zoo for his interest in this study and for providing facilities.

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(Received in June 1999; revised and accepted in July 1999)

ENTOMON 24(4): 397–398 (1999)
Short Communication No. ent.24416

Additions to the Natural Enemies of the Semilooper, *Thysanoplusia orichalcea Fab. (Lepidoptera: Noctuidae)*

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ABSTRACT: Six species of parasites one each species of predator and fungal pathogen have been recorded parasitizing/predating/fungal infection on larvae/pupae of semilooper, *Thysanoplusia orichalcea* in Bangalore (India).

KEYWORDS: *Thysanoplusia orichalcea* Fab., Parasites/Predators/pathogen.

The semilooper, *Thysanoplusia orichalcea* Fab. is a serious pest of important crops such as soybean, sunflower, niger etc., from Sept. to Nov. under dry land agro-ecosystem of semi arid tropics. The larvae causes severe defoliation especially during flowering and seed development stage leading to the reduction in seed yield. A total of eight Hymenopteran parasites viz. *Trichogramma australicum, T. japonicum, T. chilotraeae (Trichogrammatidae), Apanteles ruficrus Hal., A plutellae Kurd., A glomeratus* Linn., *Apanteles* sp. (Braconidae), *Litomatrix truncatella* Dalm. (=*Copidosoma* sp.) (Encyrtidae) and three dipteran parasites viz., *Carcelia* sp., *Voria edentata* Bar. (Tachinidae) and *Senotainia* sp. (Sarcophagidae) have been recorded (Manjunath, 1972; Brown, 1978; Taylor, 1980; Sagar & Ramji, 1990). Apart from these, the larvae were also infected by bacteria, *Bacillus cereus* var. *mycoides* (Sagar & Ramji, 1990) and Fungus, *Nomurea rileyii* (Sagar, 1984).

The material for the present study was various stages of field collected larvae. They were maintained by providing same food plants in the laboratory [28.72°C (max) and 22.65°C (min.) temperature and 96.0% (max) and 69.0% (min) relative humidity] for the emergence of parasitoids. Field observations were also made on the predator and fungal pathogen affecting the larvae. Natural enemies recorded are given in Table No. 1.

The extent of parasitism ranged from 2.0 to 13.33 per cent which is quite high and these natural enemies can be employed in the Integrated pest management programme. Among the above natural enemies the parasite, B. *lasus*, the predator C. *furcellata* and

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TABLE 1. Natural enemies of the semilooper

Natural enemies	Per cent attack
Parasites	
Apanteles ruficrus Hal. (Hymenoptera: Branconidae)	13.33
Copidosoma sp. (Hymenoptera: Encyrtidae)	10.00
Brachymeria lasus (walker) (Hymenoptera: Chalcididae)	8.33
Voria edentata Bar. (Diptera: Tachinidae)	3.50
Carcelia sp. (Diptera: Tachinidae)	2.00
Senotainia sp. (Diptera: Sarcophagidae)	5.00
Predator	
Cantheconidia furcellata (wolff.)	0.91 bug/1.35
(Hemiptera: Pentatomidae)	Larvae/m row
Fungal pathogen	
Beauveria bassiana (Bals.)	2.50

the fungal pathogen B. *bassiana* have been recorded for the first time on this insect from Bangalore (India).

ACKNOWLEDGEMENT

The authors are thankful to Dr. T. C. Narendran, Professor of Zoology, University of Calicut, Kerala and Dr. C. A. Viraktamath, Professor of Entomology and Dr. N. R. Vishwanath, Associate Professor of Microbiology University of Agricultural Sciences, Bangalore for identifying insect specimens and pathogen.

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(Received in March 1999; revised and accepted in June 1999)

ENTOMON 24(4): 399–400 (1999)
Short Communication No. 24417

First Record of *Trichogramma chilotraeae* Nagaraja & Nagarkatti on Pomegranate Butterfly, *Deudorix isocrates* (Fabr.)

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ABSTRACT: Eggs of pomegranate butterfly, *Deudorix (=Virachola) isocrates* (Fabr.) were found parasitised in the field by *Trichogramma chilotraeae* Nagaraja & Nagarkatti in June, 1998, at IIHR Farm, Bangalore. This is the first report of natural parasitism on the eggs of *D. isocrates* in pomegranate orchards by *T. chilotraeae*.

KEYWORDS: Pomegranate butterfly, *Deudorix isocrates*, Egg parasitoid, *Trichogramma chilotraeae*.

Deudorix (=Virachola) isocrates(Fabr.) (Lycaenidae, Lepidoptera) is a serious pest of pomegranate (*Punica granatum* L.) in peninsular India (Nanjan and Kumar, 1983; Shukla and Prasad, 1983; Karuppuchamy, 1994; Shewale, 1994). In order to develop a biological control programme, a search was made for its natural enemies during 1997–1998. The plant parts with the eggs of *D. isocrates* were collected and brought to the laboratory. The eggs were kept individually in glass vials ($3'' \times 1''$) to record the emergence of natural enemies.

The collection of eggs in July 1998 had yielded the adults of *Trichogramma chilotraeae* Nagaraja & Nagarkatti (Trichogrammatidae, Hymenoptera). Eight out of twenty eggs were found parasitised by *T. chilotraeae*. Number of adults emerged from a single parasitised egg ranged from 1 to 3 with as mean of 1.8. This is the first report of natural parasitism by *T. chilotraeae* on the eggs of *D. isocrates* in pomegranate orchards, though it was known to parasitise the eggs of rice and sugarcane stem borers in India. *D. isocrates* has been reported in Sri Lanka (Hutson, 1930) and ten other Indian states, but there was no record of *T. chilotraeae* on *D. isocrates* in nature. Karuppuchamy (1994) also did not observe any natural egg parasitism by *T. chilonis* (gshii) on *D. isocrates* but the same was recovered in the fields in which the laboratory reared *T. chilonis* were released in Tamil Nadu.

Adults of *T. chilotraeae* (hereafter called as pomegranate strain) obtained from the filed collected eggs of *D. isocrates* were exposed to the frozen eggs of rice moth

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Corcyra cephalonica Staint, and more than 90% of the eggs were found parasitised by *T. chilotraeae* in the laboratory. The parasitoid took 8–9 days on *C. cephalonica*. Adults lived for about 4–5 days at 28 ± 1.5 °*C* and 70–80% RH.

Several egg parasitoids including *Telenomus* sp. and *Ooencyrtus papilionis* Ashmead (Hallepponavar, 1957; Mani and Krishnamoorthy, 1996) and larval parasitoids like *Brachymeria euploeae* Westw. (Narayanan, 1954), *B. nephantidis* Gham (Narendran, 1989); *Apanteles* sp. *sauros* Nixon and *Charops obtusus* Morley (Shivale Pres. Com. 1995) and *C. brachypterum* Gupta and Maheswari (Karuppuchamy, 1994) were reported earlier on *D. isocrates* in India. But these bioagents could not be cultured easily in the laboratory for the large-scale field release in the pomegranate orchards. *T. chilotraeae* (Pomegranate strain) recorded in the present study is being easily maintained in the laboratory for more than 15 generations. Now large-scale field experiments with *T. chilotraeae* are planned for the management of *D. isocrates*.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. H. Nagaraja of Biotech International Ltd., Bangalore, for his help in the identify of *T. chilotraeae*. They are thankful to Director, IIHR for providing facilities and Mr. G. L. Pattar for his technical help in carrying out the study.

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(Received in April 1999; revised and accepted in June 1999)

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Edited, printed and published by Prof. D. Muraleedharan for and on behalf of Association for Advancement of Entomology, Department of Zoology, University of Kerala, Trivandrum. Typeset in TEX by River Valley Technologies, Trivandrum. Printed at St. Joseph's Press, Trivandrum